INDUCED MUTATIONS
FOR CROP IMPROVEMENT
IN AFRICA

PROCEEDINGS OF A REGIONAL SEMINAR
ON THE UTILIZATION OF INDUCED MUTATIONS
FOR CROP IMPROVEMENT IN AFRICA
JOINTLY ORGANIZED BY THE
INTERNATIONAL ATOMIC ENERGY AGENCY
AND THE
FOOD AND AGRICULTURE ORGANIZATION
OF THE UNITED NATIONS
IN CO-OPERATION WITH THE
INTERNATIONAL INSTITUTE OF TROPICAL AGRICULTURE (IITA)
AND HELD IN CONJUNCTION WITH AN ADVISORY GROUP
ON THE ROLE AND LIMITATIONS
OF INDUCED MUTATIONS IN PLANT IMPROVEMENT
IN IBADAN, NIGERIA, 23-27 OCTOBER 1978

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INTRODUCTORY REMARKS
Scope and Aims of the Seminar

A. Mieke
FAO/IAEA, Vienna, Austria

This seminar has been arranged with the intention to give plant breeders and plant geneticists from African countries an opportunity for refreshing and updating their knowledge in a technology that can be a useful supplement to plant breeding programmes. There are some who had been participating in training courses or study tours organized by FAO and IAEA, others who had been studying abroad under a fellowship programme. Others are holding a research contract or are counterparts in a technical assistance project. Again, there are others who know about the subject of mutation induction and the utilization of induced mutants in plant breeding only from textbooks or university courses.

We are a heterogeneous group as far as the background and expertise is concerned. We are also a heterogeneous group with regard to our interest in different plant species and breeding objectives. Some of you work with cereals, such as rice, wheat, barley, millets. Others are predominantly interested in grain legumes (such as faba bean, cowpea, soybean) or are concerned with vegetatively propagated crops. However, what is common to all of us, is that we want to learn from each other, and I think that taking a look over the fence, listening to other people's problems, considering their approaches can widen the scope, can release from pre-occupation and can lead to better, easier, faster or more economic solutions of everyone's own problems.

We all appreciate very much that the International Institute of Tropical Agriculture is the host for this meeting. The Institute not only has a world-wide reputation from its integrated research approach and the very intense work on priority crops for the humid tropics. The Institute and its staff are also known for their open mind and their critical unorthodox thinking, eager to take up ideas and new developments and to put them to work. I am very grateful to Dr. Gamble and his staff for giving the participants of this meeting an opportunity to view IITA's overall programme and to become acquainted with some of its research activities. I hope that beyond the official arrangements there will be plenty of contacts according to personal interests and I hope also that such contacts will continue and will be fruitful after the meeting, when the visitors go back to their home countries.

Let me say a few words about the sponsoring organizations. FAO and the International Atomic Energy Agency (IAEA) have a Joint Division in Vienna (Austria) which is responsible for the application of nuclear techniques towards agriculture and food. We do have 6 sections, which are indicative of the sub-programmes:
1. Animal production and health
2. Soil fertility, irrigation and crop production
3. Insect and pest control
4. Plant breeding and genetics
5. Chemical residues and pollution
6. Food preservation

In each section there are 2 - 3 professionals who are responsible technical officers for research projects, training, etc. In addition, we have a laboratory close to Vienna, where supporting work is carried out. This involves chemical analytical services for developing countries, preparation and distribution of radioactive labelled fertilizers and other chemical compounds, irradiation of seeds with gamma rays or neutrons for the purpose of mutation induction and - at a relatively large-scale - the development of entomological technology related to the so-called sterile male technique. You may not know, that our laboratory breeds several million of fruit flies each week, which after sterilisation with gamma rays, are air-freighted to Mexico for release to stop the migration of this pest towards the United States. But perhaps some of you may know, that our laboratory plays an important role in a programme on tsetse fly control that involves Nigeria, Tanzania, Ghana, Upper Volta and ICIPE in Kenya and receives financial support from Belgium, the United Kingdom and the Federal Republic of Germany.

The means of our work are manifold: There exists a technical assistance programme under which IAEA assists Member States according to their requests in a wide array of subjects. Assistance in food and agriculture constitutes about 23% of this programme, the main contributions being made towards fields such as, mineral prospection, nuclear medicine, radio-pharmaceuticals, reactor engineering, waste management, nuclear electronics, health physics and industrial applications.

In agriculture, current projects in Africa are in Egypt, Ethiopia, Ghana, Ivory Coast, Kenya, Libya, Mali, Morocco, Nigeria, Senegal, Sudan, Uganda, Tanzania and Zaire. It would lead too far to explain these in detail, but they are focussing upon water and fertiliser use of efficiency, animal health, chemical residues, food preservation and tsetse fly control as mentioned before. Activities in plant breeding play only a minor role so far.

Besides the so-called technical assistance programme (for which funds are provided under the IAEA Regular Budget, but also from UNDP, SIDA and other sources), it is worth mentioning our coordinated research programme. Through this, we link institutes in different Member States having similar research objectives, provide for regular workshops to develop joint research plans, discuss progress as well as failures, exchange experiences and results. Usually, such programmes include institutes from developing, as well as, developed countries, the former ones receiving research grants in the order of $3,000 - $6,000 per year. At present our Division handles 263 of such research contracts, 85 of which are related to plant breeding. In total,
32 research contracts are with institutes in African countries but only 4 of them relate to plant breeding. There are probably several reasons for this geographical disproportionality, but the main reason appears to be that there are only few countries in Africa that have a strong national plant breeding programme. (Although about 30 African plant breeders participated in our various training programmes during the last 10 years, only few of them have made practical use of their training).

With this background information, I come back to the seminar which we start today at IITA. Besides updating knowledge and exchanging experiences, the seminar should be an occasion to discuss the need for and the efficiency of training programmes, and other forms of assistance, as I have outlined above. A couple of years ago, I have suggested to the delegates from African countries at the IAEA General Conference the establishment of "inter-country working groups", to make better use of scarce scientific personnel through coordination of objectives, pooling of resources and sharing of results. This suggestion was enthusiastically received by the delegates, however, there are, of course, constraints in realising such a plan, and these may be an important point of discussion during this week.

I have given an introduction into the purpose and aims of this seminar and I hope that it will be useful for all participants.
Factors impeding the application of induced mutation techniques for plant breeding in Africa by J.H. Monyo

INTRODUCTION

The use of induced mutants in plant breeding is a fairly recent innovation but the number of improved cultivars and genetic variants resulting from the application of this breeding technique is steadily increasing. In Africa, there has been very little effort in this field.

It has long been known that induced mutations could be useful for the solution of specific problems where more conventional breed methods were insufficient. This has been demonstrated in rice, wheat, barley and many ornamentals. In future mutation breeding may indeed become essential for further yield, and quality improvement in some agro-ecological zones, particularly for the major cereals, where the breeding intensity has been very great.

Interest in the use of induced mutations for crop improvement in Africa started in the early seventies. The coordinated seed protein improvement programme organized by the FAO/IAEA joint division contributed greatly to the participation of African scientists in the induced mutations breeding programme. Some promising results are already apparent with rice, soybean, beans and cowpeas in East Africa and West Africa.

NATIONAL RESEARCH CAPABILITIES

Manpower resources are a major constraint in agricultural research in many developing countries particularly those in Africa south of the Sahara. The planning and organization of agricultural research in these countries is weak. Research coordination is non-existent in some countries and a team approach in the preparation and execution of research projects is lacking. For many crops and countries where there is no external technical assistance, plant breeding is a one man team! Even where external technical assistance is available plant breeding programmes which are long term in nature, have suffered most due to high turnover of expatriate personnel. As such there is no integrated approach in the generation and application of technology for development.

Induced mutants can only be beneficial to a national plant breeding programme if there is a competent team of plant breeders to use them. The success with barley at Svalöf in Sweden (such as Mari, Kristina and Mona), rice in Japan (such as Reimei) and Bangladesh (Iratom 38, etc.) demonstrate this fact.

Financial resources are also a major bottleneck for meaningful agricultural research in some African countries. There is hardly enough money to even support the conventional crop improvement methods. Chemical mutagens are not easily available and are costly. There are very few laboratories with facilities for gamma rays. Fast neutrons are not available. Seeds sent out for treatment with physical mutagens outside Africa risk being impounded by the national plant quarantine services.
Inter-regional exchange of germplasm among African countries is hampered by stringent plant quarantine regulations. While the idea is good in principle, plant breeders in some national conventional breeding programmes have had to work on a very narrow genetic base and success in obtaining new improved genotypes has been slow. In the past few years, inter-regional transport and communications have also been a major problem and still are to some extent. These factors may have contributed to the interest shown by a few plant breeders and plant geneticists in Africa to resort to induced mutation breeding.

The genetic potential of many indigenous field food crops in Africa such as millets, sorghums, terf and cowpeas has not yet been fully exploited. Conventional plant breeding methods would therefore be preferred. There has not been many examples of new varieties replacing the old land race populations. Plant breeders in Africa could for most of the food crops achieve better progress by simply exploiting the available natural genetic diversity. The conventional breeding approach would also contribute more in the improvement of indigenous vegetables, and root crops such as cassava, and sweet potatoes.

Alien introductions are quickly replacing the unimproved local cultivars in some of the wheat and rice growing areas. For instance in North Africa there has been a decline in the area under grain legumes between 1963 and 1970, mainly due to the expansion of wheat acreage consequent on the striking success of the Mexican varieties. In the absence of concerted national germplasm conservation measures, many of the adapted local cultivars might be replaced by few genetically related varieties. This progressive erosion of the local adapted germplasm base in both wild species and cultivated crops enhanced in some areas by population pressure on land resources may indeed necessitate the use of induced mutants in plant breeding in Africa earlier than would have been necessary - not only in wheat and rice but also the other crop species which are being eliminated to give way to the new introduced varieties.

**UTILIZATION OF INDUCED MUTATION TECHNIQUES**

The FAO/IAEA training courses in mutation breeding in the late sixties and early seventies helped to awaken interest among developing country scientists in the application of nuclear techniques for crop improvement. This was obviously a good thing but could be disastrous if the few African plant breeders and geneticists were to spend most of their energies on generating new genetic variants instead of exploiting to the full the available natural genetic diversity. It should therefore be stressed in regional seminars and training courses that induced mutants have a major role to play in crop improvement but only when used judiciously by a good plant breeder in a well-planned and executed conventional breeding programme.

The few plant breeders in Africa should meet more often to exchange ideas in groups organized on specific crops or researchable problems. The number is too small for subdivision into conventional and mutation breeders. They should each be aware of the advantages and disadvantages of the induced mutation approach so that they can use this tool as and when needed in their crop improvement programmes. Close cooperation among plant breeders and plant geneticists on the continent could provide the much needed team approach to the solution of researchable plant breeding problems in Africa.
INVITED LECTURES
MOLECULAR ASPECTS ON MUTAGENESIS BY IONIZING RADIATIONS AND CHEMICALS.

GUNNAR AHNSTRÖM
UNIVERSITY OF STOCKHOLM
WALLENBERG LABORATORY
S-106 91 STOCKHOLM
SWEDEN

Abstract

MOLECULAR ASPECTS ON MUTAGENESIS BY IONIZING RADIATIONS AND CHEMICALS.
Radiations and chemicals attack the DNA in various ways. Ionizing radiation seems to induce damage in both strands on the same place so that mutations arise without segregation. Mutagenic chemicals on the other hand cause base damage in one strand so that at least one DNA-replication is needed to fix the mutation. In this case replication of DNA, where the defects remain, seems to be the most critical step for production of mutations.

INTRODUCTION

Most of our information about the molecular processes involved in mutagenesis has so far been obtained from experiments with bacteria and their viruses. However, when the experiments are extended to higher systems, one usually finds that extrapolations from simpler systems have been valid. So if we want to speculate about molecular aspects on mutations in higher plants, we have to do this mostly on basis of information obtained from simpler systems.

DNA-damage induced by radiation and chemicals

Fig. 1 illustrates various points of attack on a DNA-molecule (here represented by two base pairs in the double helix).

Ultraviolet light: From a theoretical point of view, ultraviolet light has been the most important agent in use to elucidate mutational processes on the molecular level. One reason is that this agent only induces few types of changes in the DNA of which one major component, the pyrimidine dimer, usually accounts for about 90% of the biologically expressed damage. Another reason is that this defect from an analytical point of view is easy to quantify. The third reason is that in various organisms from bacteria to higher cells, a simple enzymatic repair system exists, which only acts on pyrimidine dimers. This system, photoreactivation, consists of an enzyme, which activated by visible light can use the energy of a photon to split the dimer and restore the original chemical configuration. Pyrimidine dimers are formed when the double bonds in two adjacent pyrimidine molecules are opened up and new bonds are formed linking the two molecules together. This new configuration results in breakage of the hydrogen bonds to the complementary purin bases, i.e. it affects directly the genetic code. Probably it also leads to a general distortion of the double helix.

Mutagenic chemicals: A large group of chemical mutagens are electrophilic compounds - or may be transformed to such compounds by biochemical activation - and react with atoms which possess a free electron pair. Depending on the type of mutagen, the whole or a part of the molecule may add to DNA. From a reaction point of view, the compound may react through two mechanisms, SN1 and SN2, see Manual on Mutation Breeding, Second Edition, 1977 (1) one, SN2, leading to preferential addition to N-atoms in the purine bases. Methyl methane sulfonate is one example of this class of chemicals. Ethyl nitrosourea is an example of an alkylating chemical, which reacts according to an SN1 reaction. This compound reacts with about the same rate with available oxygen and nitrogen in the DNA. The main reaction products after MMS treatment are N-7 methylguanine and N-3 methyladenine. In the case of ENU, O-ethylation of guanine and of phosphate groups become important. From Fig. 1 it is clear that MMS produce substitutions away from the base pairing region. In contrast to this ENU reacts in a way which
directly interferes with the genetic code, $\text{O}_6$-alkylation of guanine results in a loss of two hydrogen bonding sites to cytosine. The role of phosphate alkylation is, however, still unclear.

Other classes of compounds, important especially in theoretical work, are base analogues and also so called intercalating agents. For the action of these compounds we refer to Manual of Mutation Breeding (1). Ionizing radiations: These are the least specific agents. In principle any atom in the DNA can be attacked, however, some preferential sites exist. The double bonds in the bases are for example sensitive to attack. From a biological point of view, more important are the changes taking place in the backbone area, which lead to strand breaks. The most lethal lesion of all changes in DNA occurs when two strand breaks in opposite chains come close enough to break the double helical DNA-molecule in two pieces. Table 1 gives a quantitative estimate of changes in DNA induced by various agents.

<p>| TABLE 1. DAMAGE INDUCED BY A D$_{37}$ DOSE IN MAMMALIAN CELL (10$^{-12}$g DNA) |
|-------------------------------------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Double strand breaks</th>
<th>Single strand breaks</th>
<th>Base damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-rays</td>
<td>40</td>
<td>2 000</td>
</tr>
<tr>
<td>UV-light</td>
<td>10$^6$</td>
<td>10$^6$</td>
</tr>
<tr>
<td>Methylmethane sulfonate</td>
<td>2 000</td>
<td>10$^6$</td>
</tr>
</tbody>
</table>

DNA repair processes in cells

Cells have now the ability to repair damage to their DNA. That cells need to remove the damage can be exemplified by bacterial mutants, which lack the ability to repair UV-induced pyrimidine dimers. In those bacteria 1 dimer per bacterial genome is lethal.

All cells possess a range of systems which are counteracting the action of mutagenic agents on their DNA. The simplest system is that operating on UV-induced pyrimidine dimers, photoreactivation, where one enzyme activated by visible light splits the dimer and restores the original DNA-bases. Photoreactivation exists in bacteria as well as in higher cells.

Excision repair: A more general system for damage corrections is excision repair where the damaged subunits are taken out from the DNA and replaced with new material. The basic enzyme set up for this process consists of a damage specific endonuclease which recognizes the damage and make a nick in the strand adjacent to the damage. A DNA-polymerase can now bind, the damaged material is peeled off and cut off by exonuclease activity, at the same time as a resynthesis occurs. Finally ligase action close the gap between the newly inserted material and the old strand (Fig. 2). Large variations occur in the excision repair process, for example with regard to how extra material is removed together with the damaged nucleotide. In bacteria thousands of nucleotides can be removed per site under repair, in mammalian cells the mean is around 100. Plant cells probably have a very economic excision repair since one cannot find much incorporation when radioactive nucleotides are used for repair incorporation.

Post replication repair: In case the damage is not removed before the cell enters the S-phase - the DNA replication stage - alternative ways exist which will reduce the harmful effects of DNA-defects. This is exemplified in Fig. 3. The DNA synthesis approaching a defect in one of the strand makes a halt. After some delay the synthesis is taken up again on the other side of the defect leaving a single stranded gap. This gap may then be filled by a post replication repair process which may involve alternative steps, (a) a simple resynthesis ignoring the defect, (b) a branch migration releasing a single stranded region from the other strand which could be used to synthetize over the damage or (c) a recombinational process, where material from the sister strand is used to fill the gap. In all cases the defect remains in the DNA but can again be handled by the excision repair system.
How does mutations arise?

Several different mechanisms are possible. The simplest case is direct mispairing where a base is altered from the aminoform to the iminoform (adenine and cytosine) or from the ketoform to the enolform (thymine and guanine). Certain chemical reactions with bases in the DNA may force such rearrangements (or increase the probability of a base existing in an unusual configuration). Also incorporation of modified bases for example 5-bromouracil, which replaces thymine, appear to promote mutagenic tautomerisations. This type of event causes transition i.e. a C-G pair is changed to A-T, or vice versa. This type of mutations does not involve active participation of repair enzymes.

Another type of mutations, frameshift mutations, are believed to involve enzymes. The frameshift means that base pairs have been deleted or added in such a way that the whole message after the error is out of phase. One visualizes the frameshift to take place according to the following sequence; single strand breaks, eventually extended to a gap. This is followed by local melting i.e. separation of the strands in the double helix. The annealing of the denatured regions may then give rise to wrong pairing, a few bases looping out. Then repair synthesis closes the gap while preserving the extrahelical loop. Replication and segregation then follows. It is interesting to note that frameshift mutations arise in the vicinity of strand discontinuities and in the case of local base sequence redundancy.

Missrepair mutagenesis: Mutagenesis where repair enzymes play an active role seems to be a major mechanism (2). Error-free and error-prone mechanisms exist and the general point is that mutants defective in error-prone repair are more sensitive towards mutagenic agents but the mutability is decreased. The error-prone repair system can be regarded as a life saving emergency system but in saving their life the organisms has to pay in form of more mutations.

The normal excision repair system discussed earlier (Fig. 2) is regarded as a largely error free system, in principle it should not be more subjected to errors than the ordinary semiconservative DNA-synthesis. The errors are then more likely to occur when defects remaining in the DNA are going to be replicated.

A model for how errors can be introduced has been presented recently and has also experimental support (3). The DNA-polymerase which is inserting new nucleotides during DNA-replication has a proof-reading part, an exonuclease activity, which immediately removes a newly inserted nucleotide which does not base pair. This means that when the polymerase encounters a defect where base pairing properties are lost, for example a pyrimidine dimer, it is stopped by its own proof-reading activity which does not accept any errors. The replication can, however, be reinitiated on the other side of the damaged side leaving a gap in the newly replicated strand. This gap may now be filled in different ways as discussed above. One way of filling the gap is by the function of an induced DNA-polymerase which lacks the proof-reading exonuclease activity. This enzyme may now ignore the defective bases in the complementary strain and mutations are created by incorporation of wrong bases.

Processes responsible for mutation by different agents

Much of the earlier work on the mechanisms of mutations were done on bacteriophages (4). In this type of work a number of chemicals were used to induce mutations. Mutants were collected, multiplied, and treated with each one of the compounds used to produce mutants. Screening was done for reversions, i.e. back mutation to the original configuration of the gene under investigation. By this way it was found that base analogues mainly caused base pair substitution and it was also possible to show that bromouracil predominantly induced changes in one base pair from GC=AT. Aminopurine give a majority in opposite direction while nitrous acid seemed to give both ways with the same frequency. Hydroxylamine induced as expected only transitions GC=AT. Reversion tests showed that ethyl ethane sulfonate mainly introduced GC=AT shifts but also mutations which were not of the base substitution type.

Dyes of the acridine series, proflavin and 5-aminoacridine are mutagenic in phage but less so in higher systems. The mutations induced by these so called intercalating agents could not be reverted by the class of chemicals discussed above. These agents have been found to induce deletion or insertion of a base-pair causing complete misreading of the message after the defect (frame shift). The same type of experiments have also been performed in mammalian cells in culture using cloning techniques and different selective
media. One system in common use is mutation to azaguanine resistance, an x-linked biochemical character which is expressed when the function of an enzyme is destroyed. The reversion tests performed have in principle shown the same pattern as phage experiments, namely that two types of mutations can be positively identified, base-pair mutations and frame shifts. Chu (5) gives details of a mutation study employing a number of alkylating agents, an intercalating agent IC-170, a compound which induces a bulky substituent in DNA, N-HCO AAF and X-rays. He finds that mutants induced by MMS, EMS and MNNG are caused by base-pair substitutions to 30%. 20% reverted only spontaneously and 50% did not revert at all. These later classes of mutations are of unknown type. No frame shift mutations were induced by these chemicals. The spontaneously arisen mutants showed about the same distribution between classes. On the other hand N-HCO-AAF and X-rays gave a high frequency of frame shift mutations. It is interesting to note that practically all mutants recovered from high dose X-ray treatment were of the non-reverting type.

It is also possible to investigate a mutation on the protein level, first if a protein is made at all and secondly if it is functional. Mutants induced by chemicals giving base pair substitutions usually still have a functional protein made. Usually one amino acid is substituted and this may reduce the activity or change specificity. In contrast to this, mutants arising from treatment with acridine compounds do not make functional proteins. The reason for this is as discussed earlier that a deletion or addition of a base pair will lead to a complete misreading of the message.

Mutations by ionizing radiations: It is known from several systems that mutations induced by ionizing radiation arise without segregation, i.e. both daughter cells are mutants. This is in contrast to the action of chemical mutagens. In this context it may be pertinent to discuss the effect on chromosomal level since this may give an indication of the differences involved. Ionizing radiations induce aberrations of chromosome type if cells are irradiated in G0 or G1 stage of the cell cycle and chromatid aberrations if irradiated in G2 immediately in the following mitosis. UV and chemicals induce chromid aberrations for treatment in G2 and no aberrations after treatment in G2 under the same conditions of scoring. For chemical mutagens it is necessary to pass a S-phase-DNA-replication to obtain a chromosome aberration, X-rays act independently of DNA-synthesis. The unique type of damage induced by X-rays (Table 1) is double strand breaks, i.e. the G1-chromosome or G2-chromatid can be broken and reconstructed without involvement of DNA-replication. After treatment with agents that only induce damage in one strand it would in principle be possible to produce double-strand lesions by action of enzymes. This does not, however, seem to happen and a DNA-replication is required to spread the damage to bothstrands of the DNA-helix.

It seems that mutations follow the same pattern, which would indicate that mutation by ionizing radiation is caused by an event where information is lost from both strands in the same place. This damage need not be a double strand break it could also involve destruction of the two bases in a pair primarily leaving the backbone intact.

In this context it is interesting to note that high LET radiations on hamster cells show that double strand breaks increase with increasing LET to the same extent as chromosome aberrations and cell killing. Single strand breaks and base damage on the other hand decrease with increasing LET.

High LET radiation now also induce a correspondingly higher mutation frequency in Chinese hamster cells (6).

The best examples of high LET effects on mutations comes, however, from plants where RBE-values for mutations are found to be as high as 50.

Mutagenic chemicals: If a mutation by ionizing radiation can be formed because information is lost in both strands on the same place, the situation seem to be much more complicated in the case of UV and mutagenic chemicals. The reason for this is firstly that these agents only create damage in one strand, i.e. the information for correction of damage is still there, and the cell has multiple ways of correcting it. The correction mechanisms can operate on different levels, in competition or cooperation with the DNA-replication. How complicated the situation may be is exemplified below. After treatment on dividing cells excision repair start to remove defects in the DNA. If now DNA-replication encounters remaining lesions several things may happen. If the defect base can pair normally the DNA-replication probably continues undisturbed. Otherwise it seems that replication is halted at the defect and reinitiated on the other side leaving a single stranded gap of about 1000 nucleotides. The gap may now be filled by post replication repair, of which several types probably exist. Some of them may use information in
the sister strand to incorporate the correct information opposite the defect, while other error-prone systems may have to incorporate bases at random. In each case the original defect is left and can now again be subjected to excision repair. In case the post-replication repair was error-prone a mutation will now be fixed by excision repair or further replications.

We have studied the effects of ionizing radiation, ultraviolet light and a number of methylyating and ethylating chemicals on the ability to induce post replication defects in DNA (Fig. 3) and induce mutations in Chinese hamster cells. There is a close correlation between the ability to induce a replication gap in the DNA and mutation induction. This indicates that direct mispairing i.e. DNA-synthesis runs uninterrupted over a defect incorporating a wrong base is not the mechanism in higher cells. The interpretation is further that alkylated guanine does not induce a base pair substitution because it pairs with thymine instead of cytosine. It need not pair at all with the inserted base, the wrong base is inserted at random and is only not removed by the proof reading capacity of the repair system. One could even imagine that the presence of an error-prone repair system introduces errors on places where the information is intact. In such a case the main effect of the mutagenic agent is that it has induced the error-prone repair system.

CONCLUSIONS

From experiments with a variety of organisms it seems that the mutational process is much more complex in the case of agents which induce single-strand damage, for example alkylating agents compared to ionizing radiations.

The complexity of repair phenomena when acting in cooperation or competition with each other and the DNA-replication after treatments with chemical mutagens explains the variability in results of a certain mutagen applied to different organisms and conditions. In this respect ionizing radiation seem to be much more predictable with respect to biological response. This does not mean, however, that the spectrum of mutagenic events is broader after chemical mutagens compared to radiations. On the contrary, reversion experiments on mammalian cells show that alkylating agents induce a spectrum of mutations similar to that arising spontaneously. Ionizing radiation on the other hand, induce a type of deletion-addition effect which is absent in material treated with alkylating agents.

Figure 1. Major sites of reaction between mutagenic agents and DNA.

Ionizing radiation

Ultraviolet light

MMS, ENU

ENU additional sites
Figure 2. Excision repair of base damage induced by a mutagenic agent.

- a) base damage
- b) A damage specific endonuclease recognizes the defect and cuts the strand.
- c) DNA-polymerase fills in the gap at the same time as an exonuclease removes the damaged piece of DNA.
- d) Ligase repairs the last single strand gap.

Figure 3. DNA-replication has started with defect still remaining in the DNA. A gap is left in opposite of the defect. This gap is filled in a process which is much slower than the DNA-replication.
Economic aspects of using induced mutations in plant breeding

R.D. Brock* and A. Micke
Joint FAO/IAEA Division of Atomic Energy
in Agriculture, IAEA, Vienna

Abstract

Economic aspects of mutation plant breeding are considered in relation to basic principles of mutation induction and selection, when to use mutations in plant breeding programmes and mutation breeding strategies.

The following basic principles of mutation plant breeding are outlined:

- A mutation is induced in a single cell and expressed in the progeny from that cell.
- Expression of a mutation is influenced by its genetic nature and the breeding system of the organism.
- The mutation frequency determines the number of cell progenies that have to be examined to detect a particular mutant.
- Random mutations in quantitatively inherited characters generally result in an increase in variance and a shift in the mean.

Mutation rates for various types of mutations have been estimated and population sizes for mutation plant breeding experiments calculated. Based upon these principles the potential for using induced mutations in plant breeding and some breeding strategies are outlined.

Introduction

Economic considerations require, first, an understanding of basic principles, followed by decision making and then the choice of a strategy which will make maximum use of the available resources to ensure a satisfactory return in relation to investment.

Unfortunately, plant breeders do not often think enough in terms of the economy of their programmes. This is surprising, for plant breeding is expensive of time, money and resources. Röbelen [20] estimated that the development of a variety takes 10 to 15 years and costs around one million US dollars. This is a lot of money, but the time is also important. Remember that 10 to 15 years is a very large proportion of the working life of a plant breeder. Hence, serious consideration should be given to the economic aspects of plant breeding. Such considerations are also advisable for the use of induced mutations in plant breeding. We are not planning to present a cost benefit analysis. This is a very difficult task and may be attempted by others. We will only touch some principles and general features, which should be taken into account in the planning stage.

*Permanent address: CSIRO, Division of Plant Industry
Canberra, Australia
We will discuss these under the three headings basic principles, decision making and choice of strategy. You are all trained and experienced plant breeders, so we don't need to emphasize the importance of a clear definition of your breeding objectives. This applies to all types of breeding programmes. We will concentrate upon the basic principles of mutation induction, expression and selection and upon the plant populations that have to be examined to ensure a high probability of success.

Some of the following will sound rather trivial to the experienced, but we want to stress that an understanding of these principles is necessary before you can decide whether to start a programme, and whether to use mutation or hybridization, or both.

If your decision is to induce mutations, these same principles together with your knowledge of the species, the breeding objective and the resources you have available will enable you to choose the strategy most likely to be successful.

**SOME BASIC PRINCIPLES OF MUTATION INDUCTION, EXPRESSION AND SELECTION**

1. **A mutation is induced in a single cell and carried only in the progeny from that cell.**

   In the case of single cell organisms, e.g., bacteria, this presents no problem for, after mutagenesis, separate single cells can be grown to provide single colonies which can be subjected to selection. However, in multi-cellular organisms, such as higher plants, the organ that is exposed to mutagenesis, e.g., seed, is usually also multi-cellular and hence, the progeny from a mutant cell will represent only a portion of the resultant first generation growth, i.e., the $M_1$ plant will be chimerical. Hence, selection of mutants cannot normally be practised in the $M_1$ generation. There are some special situations where treatment can be applied to single cells such as pollen grains, eggs, or bud initials in certain vegetatively propagated species [7] where mutants which express can be selected in the $M_1$ generation. More usually, the $M_1$ plant has to be self-fertilized and mutants sought in the $M_2$ generation.

   For our purpose, it is important to know the number of cells in the multi-cellular organ exposed to the mutagenic treatment which contribute to the next generation, to the formation of seed, the "genetically effective cell number" (GECN) [10]. This number has been estimated for seeds of a number of species (see [3,10] for references) and is usually between two and ten. As a simplification in the subsequent calculations the GECN will be assumed to be five.

2. **Expression of a mutation is influenced by its genetic nature and the breeding system of the organism**

   Dominant mutations will of course express as heterozygotes and hence, can often be detected in the $M_1$ generation; as chimeras following treatment

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of a multicellular organ such as a seed or bud, or as homogeneous individu-
als following treatment of single cells or regeneration of a plant from only one of the treated cells.

Recessive mutations will only be expressed when homozygous or hemizygous. When homozygous dominant diploids are mutated, recessive mutants will only be expressed after self-fertilization or sib-mating. The earliest generation at which this can occur is the $M_2$ generation following self-fertilization of $M_1$ plants. In self-incompatible species, sib-mating of $M_2$ individuals will permit expression in the $M_3$ generation. Recessive mutants induced in heterozygotes may express as homozygous tissue of chimeras in the $M_2$ generation or as a non-segregating plant progeny in the $M_2$ generation, if the mutated allele is identical with the pre-existing non-mutated one in the same locus.

3. The mutation frequency determines the number of cell progenies that have to be examined to detect a particular mutant.

Assuming a mutation rate ($\mu$) and setting a level of probability for the occurrence of at least one mutation ($p$), the number of treated cells that have to be examined ($n$) can be calculated for various mutation rates (Table 1) using the formula,

$$n = \frac{\log (1 - p)}{\log (1 - \mu)}$$

Estimating the mutation rate that can be expected for particular mutations is more difficult. Mutation rates vary greatly depending upon the locus studied, the nature of the mutational event and the mutagenic treatment.

For example, gross chromosomal aberrations affecting many loci are induced with high frequency, but these are rarely to result in desired mutations in highly selected organisms. Single locus changes with few or no other accompanying genetic changes are the most desirable, but difficult to accurately classify. The determination of a mutation as a true intra-locus event is dependent upon precise genetic tests, requiring the availability of closely linked flanking markers together with studies of fertility of the homozygous mutant and genetic transmission of the mutated gene. Such conditions are available for only a few well-studied genetic organisms and very little reliable data is available for higher plants.

Brock reviewed the available data for X- and gamma-ray induced mutations in a range of eukaryotes and found induced mutation rates ranging from less than $7 \times 10^{-6}$ per locus per cell in Neurospora, Drosophila and maize, where the most stringent tests for intra-locus mutation are available, to a maximum value of $5 \times 10^{-4}$ per locus per cell for other loci in maize where only widely spaced flanking markers are available [6].
It, therefore, appears reasonable to assume a mutation rate of less than $1 \times 10^{-4}$ per locus per cell induced by a medium dose of X- or gamma-rays for intra-locus recessive mutations in higher plants.

Less data is available for specific-locus mutation induced by chemical mutagens but rates ranging from $1 \times 10^{-5}$ to $3 \times 10^{-3}$ per locus per cell induced by ethyl methane sulphonate (EMS) have been reported. Direct comparisons of the mutation frequencies at "specific loci" in plants induced by ionizing radiations with those induced by a number of chemical mutagens reveal that where EMS and ethylene imine (EI) induce higher rates than ionizing radiations, the increase is usually only two- to three-fold. The highly effective mutagen, sodium azide, has not yet been compared with ionizing radiation for "specific locus" mutation and one might expect a somewhat larger increase with this mutagen.

How representative are these specific loci of the loci which we wish to mutate improve plants? Wide differences in the spontaneous as well as the induced mutation rates at different loci have been reported [11, 23]. It seems reasonable to assume that the loci most frequently studied in genetic experiments are not only those with readily detectable effects, but also those which mutate more frequently than normal.

This view is supported by comparisons of the mutation rates at seven specific loci in mice, which have been so extensively utilized in mutation studies, with the rates for recessive lethal and visible mutations [13, 21]. The spontaneous mutation rate for recessive visible mutations occurring at 26 loci was considerably lower than that for the specific loci used in the genetic studies ($0.67 \times 10^{-6}$ c.c. $8 \times 10^{-6}$/locus/gamete [21]. Similarly, Lyon et al. [13] noted that the induced mutation rate to recessive lethals was about 1,250 times, and to recessive visibles about 200 times, the specific locus rate. Lyon estimated the number of loci capable of mutating to recessive lethals at about 10,000 [12] and there are undoubtedly more than 200 loci capable of mutating to recessive visibles, suggesting that the specific loci are more mutable than the average loci [23]. Hence, if we accept $1 \times 10^{-4}$ per locus per cell as an average rate for recessive intra-locus mutations at specific tester loci, induced by ionizing radiation, there are indications that this rate may be too high, by factor of up to 10 fold, to be representative of "average" loci. However, this could be compensated by the higher effectiveness of some chemical mutagens. Hence, in subsequent calculations an induced mutation rate for single gene recessive mutation of $1 \times 10^{-4}$/locus/cell will be assumed as a current 'best estimate'.

If this rate is assumed for recessive single locus mutations, the expected frequency of mutants which can result from more than one locus can be calculated. For example, "high lysine" mutations in barley are known to occur at a number of loci [8] and if 10 loci are assumed, an expected mutation frequency of $1 \times 10^{-3}$ would result. This is close to the frequency observed by Doll et al. [8]. Similarly, if 500 loci are assumed conditioning
chlorophyll synthesis, and that mutation of any one locus will induce a chlorophyll deficient mutant a mutation frequency of $5 \times 10^{-2}$ would be expected. This is also comparable with the observed mutation frequencies.

Gustafsson has pointed out that 69 loci are involved in the determination of waxiness in barley. Other studies of Gustafsson and his colleagues have shown that there are at least 26 loci determining the short, stiff-straw, erectoid mutants in barley [14]. While no estimates have been made of the number of loci determining flowering or maturity time, early and late flowering mutants occur with high frequency so several loci can be assumed too.

To know the rate of mutation that can be expected from recessive to dominant alleles would be most valuable, however the low frequency of occurrence of dominant mutations has precluded reliable estimates at specific loci. Reverse mutation rates are greatly influenced by the nature of the forward mutation and in higher organisms the vast majority of 'revertants' are the result of mutation at some other (suppressor) locus rather than true revertants.

Gaul (personal communication) estimated the proportion of dominant viable mutations in barley to be 6% of a total of 119 mutants reported [9, 15, 17, 22].

About 75 loci are known in mice at which dominant visible mutations have occurred [23]. Using this number of loci, an average spontaneous frequency of about $1 \times 10^{-7}$/gamete/locus for dominant mutation is calculated. This compares with the average spontaneous rate of seven specific recessive loci of $8 \times 10^{-6}$/gamete/locus, indicating that 1 dominant mutant occurs for every 80 recessive mutants, i.e., 1.25% dominant mutants. Similar calculations for induced mutations indicate an average rate for dominant visibles of $6.2 \times 10^{-9}$/locus/gamete/rad compared with an average rate for recessive specific locus mutations of $2 \times 10^{-7}$/locus/gamete/rad (after [23], i.e., 3.1% dominant mutants. Batchelor et al., [1, 2] have reported mutation frequencies for dominant visibles and recessive specific locus mutations in mice induced by gamma rays and fission neutrons. Again, assuming 75 loci for dominant visibles and correcting for homozygous lethality, values for the percentage dominants range about 1 to 2%.

Considerable uncertainties exist about these estimates for there is no certainty about the number of loci concerned, nor that the loci are comparable as regards their mutability. None of the studies involve chemical mutagens which would be expected to induce a higher frequency of intra-locus mutations than induced by ionizing radiations. Brock earlier estimated [5] $1 \times 10^{-5}$ as the induced rate for dominant mutations but this should be regarded as an upper estimate, the more likely value being between $10^{-5}$ and $10^{-6}$ (Table I).
4. Random mutation in quantitatively inherited characters generally result in an increase in variance and a shift in mean.

The response of quantitatively inherited characters in self-fertilizing species to random mutation will depend on:
- the number of genes involved,
- the relative proportion of genes with positive and negative effects,
- the extent to which the genes of the parental genome operate as a balanced set, and
- correlations due to linkage or pleiotropy

The simple expectations of this hypothesis are that random mutations will increase the variation of all quantitatively inherited characters in the $M_2$ generation and shift their means away from the direction of previous selection. Effects due to altered genetic balance and genetic correlations will disturb this simple pattern somewhat. However, the general consequences will be that effective selection responses should be possible in either direction. If no selection is applied after the induction of mutations, the average effect will be a regression of the mean and a reduction of fitness for all adaptive and previously selected characters.

These findings have important consequences for the use of induced mutations in plant breeding programmes. Firstly, inducing mutations followed by proper selection will result in improvement of quantitatively inherited characters. However, if selection is restricted to only one or a few specific characters the resultant selections will probably carry alterations in other genetic systems, and on average these alterations will be deleterious. Even highly advantageous single gene mutation, such as giving better disease resistance are usually found to be associated with altered genetic backgrounds. In such situations the mutant must be used in a hybridization programme or induced a sufficient number of times so that selection for all other important characters can be practised. Even when selecting mutants of quantitatively inherited characters, which occur with high frequency, populations must be large enough to permit selection for all of the important characters.

POPULATION SIZE FOR MUTATION PLANT BREEDING EXPERIMENTS

Combining the estimated mutation frequencies with knowledge of the breeding system and the expected type of mutational change, the required minimum numbers of $M_1$ plants and $M_2$ progenies can be calculated (Table I).

Dominant mutants can be detected in the $M_1$ generation under special conditions, so in these cases the number of cell progenies, $n$ from Table I, (230,000 to 2,300,000) represent the minimum number of $M_1$ plants that have to be screened. Where screening is delayed these numbers of cell progenies are required for screening in the $M_2$ generation.
Recessive mutations cannot normally be detected before the \( M_2 \) generation. Hence, the values of \( n \) in Table I indicate the minimum number of cell progenies that have to be examined. If the mutagenic treatment has a 50\% lethal effect, the number of treated cells required to provide the \( M_2 \) families is \( 2n \). For seed treatment, if we assume five genetically effective cells per seed, \( 2n/5 \) \( M_1 \) plants are required. Accordingly ca. 10,000 seeds would be the minimum number.

The number of plants per \( M_2 \) family required for the expression of the recessive mutation is determined by the segregation ratio which should normally be 1/4 but decreases to 1/20 if the GECN is about five. However, as the number of plants that can be grown and subjected to selection is normally limited, a compromise is usually necessary between the number of progenies of treated cells that are examined and the size of the \( M_2 \) progenies. Rédei [18] and Rédei and Acero [19] have reviewed the numerous considerations influencing the optimum size of the \( M_2 \) families and also the proportion of effort that should be devoted to the \( M_1 \) and \( M_2 \) generations. In conformity with most other authors, they conclude that best efficiency is achieved by large \( M_1 \) populations and small \( M_2 \) families.

The ultimate in this respect is a single \( M_2 \) plant tested from each \( M_1 \) plant or treated cell. This technique has been successfully used by Doll et al., [8] for the selection of "high lysine" mutants in barley. Although it does require a four-fold increase in the size of the \( M_1 \) population to compensate for the segregation of recessive mutations, the \( M_1 \) is normally the generation that can be produced at least cost and effort [19]. Single seeds can be harvested and bulked without identification of the \( M_1 \) plant origin and a duplicate sample for separate screening can be obtained at very little additional cost.

Examples are given in Table II of the numbers of \( M_1 \) and \( M_2 \) plants required for a typical experiment with cereals where one spike has been assumed to represent one treated cell. Obviously, smaller populations are needed if more than one locus can be mutated to produce the desired mutant.

**WHETHER TO USE MUTATIONS OR SOME OTHER APPROACH IN PLANT BREEDING**

Any proposal to use induced mutations in plant breeding must consider the likelihood of success when compared with other techniques and the effort required to obtain the desired genotype. The likelihood of success cannot be predicted, but may be considered in relation to the genetic control of the character to be improved and the breeding system of the species.

For simplicity, the alternative breeding systems and types of genetic control are considered as discrete systems. This is, however, not strictly correct. While most species are predominantly self- or cross-fertilizing and predominantly sexually or asexually reproducing, many intermediate situations occur. Similarly, while some traits are simply inherited, the phenotypic expression of genes with large effect can be modified by other genes; and mutations of large effect sometimes occur in traits usually assumed to be inherited in a quantitative fashion.
1. Simply inherited traits

The choice between induced mutations and gene transfer in the case of simply inherited traits is largely determined by the ease with which the gene can be mutated, compared with the ease with which it can be incorporated from another genotype:

e.g., the induction of a recessive mutation is a much more likely event than the induction of a dominant one. This fact, plus the ease of intraspecific transfer of a dominant gene by conventional genetic methods, favour hybridization breeding where the desired characteristic is known, or presumed to be conditioned by a dominant gene. Unfortunately, the breeder too often has to make his decision about the strategy knowing only the desired phenotype but little or nothing about the genes eventually involved.

In case traits are known to be resulting from recessive genes, the induction of mutations is of course more worthy of consideration. If the gene is available in a genotype closely related to the agricultural cultivar, transfer of the gene by back-crossing would normally be favoured as the method more likely to prove successful. However, there are examples of rapid adjustment of single undesirable characteristics by means of mutation. If close linkage with undesirable characters or undesirable pleiotropic effects are found to be involved in a gene-transfer programme, or if transfer of the desired gene involves inter-specific or inter-genetic crossing, induced mutations may be the preferable technique. If there is no known source from which the gene can be transferred, induced mutations are, of course, the only possibility and the decision to initiate a programme based on induced mutations depends largely upon economic considerations such as the importance of the objective and the cost of screening for the desired mutation.

Mutations are particularly efficient aids to the domestication of wild species. Evolution under natural conditions results in the accumulation of genes advantageous to the survival of the wild plant and for its reproduction. Many of these genes are disadvantageous in agricultural situations. Where they occur as dominant alleles in the wild plant, their activity can be destroyed by deletion, or altered by mutation to recessive alleles, or suppressed by mutation of other modifier genes. Removal or suppression of undesirable features such as shattering seeds, breakable spikes, dehiscent fruits, a toxin, spines, or thorns, is the first step in the adaptation of a plant to man’s use. With all of our older agricultural species, this has already been accomplished, but it remains of importance for the introduction of new species into agriculture. For example, many of the potentially valuable high protein grain legumes have undesirable morphological as well as anti-nutritional factors and toxins limiting their use.

2. Quantitatively inherited traits

These characters are controlled by the interaction of many genes each then having only a small effect. In these situations, the efficiency of
selecting the desired mutant is generally lower than for specific characters which are controlled by a single gene, but this is largely offset by the increased frequency of mutants resulting from the greater number of genes involved. Alternative sources of variability, and the price to be paid in terms of alteration to the background genotype are the most important considerations to be taken into account in deciding whether to use induced mutations to improve characters, such as yield, maturity time, adaptability, etc. [4].

In the situations where the species under consideration has not been closely adapted to the environment by intensive plant breeding, substantial variation is likely to be generated by either mutation or intervarietal hybridization. Consequently, the choice between methods of inducing variability will be largely determined by the extent to which the background genotype will be changed by the different methods. Where mutant identification is highly efficient, e.g., early flowering, induced mutations appear to be an attractive proposition, for many mutants can be obtained and selection for other characters can be practised. On the other hand, if the selection is difficult and expensive, e.g., yield, hybridization between high yielding varieties would be favoured because high yielding segregants would less likely be deficient for some important productivity characters.

In situations where the species has been closely adapted to the environment by intensive plant breeding, further intervarietal hybridization of high-yielding, well-adapted genotypes generates very limited variability. In these situations substantial variability can only be generated by wide hybridization or by mutation. Both methods will upset adaptation and where the effect of inducing mutations is likely to be less drastic, it would be the preferable method.

3. Self- and cross-fertilizing species

In general, induced mutation techniques offer less prospect for the improvement of cross-fertilizing species than for self-fertilizing species. This is partly because of the difficulty of selecting, incorporating and maintaining recessive mutations in such populations and partly because the plant breeding problems in cross-fertilizing species are more often problems of handling existing variability than lack of variability per se. However, it is the kind of genetic variability that counts, not its amount and therefore plant improvement programmes with cross-fertilizing species have successfully utilized variability induced by mutagenic agents [16, 24].

Where a lack of variability exists for specifically desired simply inherited traits, the basis for choosing between induced mutations and hybridization is essentially the same in self- and cross-fertilizing species. However, the consequences of the failure of recessive alleles to express in cross-fertilizing systems without forced selfing or sib-mating must be taken into account.
Compared with the situation with self-fertilizing species, relatively few experimental studies involving mutation and selection for quantitatively inherited characters are available with cross-fertilizing species. Experiments with maize and poultry have shown that mutagenic treatment results in a sharp depression of the mean of the character under study. An enhanced selection response can be achieved in the mutated population, but the selections from the mutated populations have rarely exceeded those from the unmutated controls [14]. In view of the fact that lack of genetic variability is rarely the factor limiting improvement of quantitatively inherited characters in cross-fertilizing species, the use of induced mutations in these situations cannot be generally recommended unless it has been clearly established that naturally occurring variability has been fully utilized.

4. Vegetatively propagated species

Vegetatively propagated species fall into two main categories. Firstly, those which are capable of sexual reproduction, but are commercially propagated vegetatively, e.g., many horticultural and ornamental species. These species are generally highly heterozygous and are often polyploid or aneuploid. The variability generated by crossing is so great that there is little chance of selecting for improved types among seedling progeny and at the same time retaining the general characteristics of the variety. Plant improvement has depended very largely upon the selection of naturally occurring mutants (sports). Consequently, techniques which increase the frequency of mutations should be of great value particularly where they can be combined with adventitious bud formation to avoid the production of chimerae [7]. This is already proving so and many new ornamental varieties, produced as the result of the induction of mutations have been released [7]. Restrictions imposed by the preponderance of recessive mutations and the failure of such mutations to express unless homozygous, are largely offset by the heterozygosity of the cultivars. The largely deleterious nature of mutations and the detrimental effects of mutations occurring in the background genotype are of less relevance in ornamental species where novelty is of value in itself and "yield" is not as important as it is in agricultural species. However, these factors remain important in other horticultural species used as food or feed crops.

The second category of vegetatively propagated species are the apomicts and sterile forms. In obligate apomicts, hybridization cannot generate any variability. In such situations induced mutations are the only available method for generating variability, but it should not be assumed that obligate apomicts occur frequently and an exhaustive search for sexual forms or facultative apomicts should be made before assuming that a species is an obligate apomict.
CHOICE OF STRATEGY IN MUTATION BREEDING

From what has already been said it is obvious that the mutation breeding strategy must be matched if possible with the genetics of the breeding objective, the breeding system of the species, the end use of the mutants and the available resources. This presents many complexities and while some strategies have already been mentioned, this is better the topic of individual discussion and detailed study of the Manual of Mutation Breeding [14]. Hence, we will offer only a few generalizations.

1. **Mutagenic treatment**

   At least two different mutagens, e.g., X- or gamma-rays and EMS or another highly effective chemical mutagen, should be used at doses which permit about 50% of the treated seeds to produce $M_2$ progeny. In our opinion, provided that the treatment is effective in inducing mutations, this phase is less important than the method of selection.

2. **$M_2$ generation** should be grown so that small $M_2$ progenies are produced. Harvest should be of individual seeds, pods, spikes, fruits or plants. Rarely is bulk harvesting satisfactory with the exception of single-seed-bulks.

3. **$M_2$ and subsequent generation**

   Chlorophyll mutations can be used as an indication of the effectiveness of the mutagenic treatment. The method and generation of screening must be determined for each individual situation. Examples of mutation breeding strategy are being discussed during this seminar and can also be found in the Manual on Mutation Breeding, published by IAEA, Vienna in 1977 [14].

**Summary**

1. Plant breeding is an expensive venture. Therefore, it is important that the breeder plans carefully his programme. This also applies to the use of induced mutations.

2. Where there is a real choice between different breeding approaches, such as hybridization or mutation induction, an estimate of labor and time investment versus the likelihood of success is worthwhile, although it is generally difficult to predict the outcome of a breeding programme.

3. When hybridization is not an alternative (as in apomicts), mutation induction may be the only method. However, also here, the importance of the breeding objective should be valued against other solutions.
4. When plant breeding progress is limited by lack of suitable germ plasm, mutation induction again may be the method to create genetic variation. The breeder will have to base his decision upon a consideration, whether the objective is important enough to justify the effort. The same economic consideration should eventually apply regarding the only alternative namely, the search for variants in centers of diversity. Fortunately, the breeder has rarely to worry about the costs of germ plasm collection.

5. Estimates of minimum population size for mutation breeding were given, which should help the breeder to make a more realistic cost estimate.

6. As far as strategy of mutation breeding is concerned, only some advise of general nature has been given. But many points have to be considered, so that advise—to be useful—necessarily has to be specific, depending upon plant species, breeding objective, available screening technique, etc. The Joint FAO/IAEA Division in Vienna will make available such advise upon request.

REFERENCES


Table I. Number of cell progenies to be examined for various mutation rates (\(1/u\)) and probabilities of occurrence (\(p\)).

<table>
<thead>
<tr>
<th>Mutation rate ((1/u))</th>
<th>No. of cell progenies (n)</th>
<th>Type of mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p = 0.90</td>
<td>p = 0.99</td>
</tr>
<tr>
<td>(1 \times 10^{-2})</td>
<td>230</td>
<td>460</td>
</tr>
<tr>
<td>(1 \times 10^{-3})</td>
<td>2,300</td>
<td>4,600</td>
</tr>
<tr>
<td>(1 \times 10^{-4})</td>
<td>23,000</td>
<td>46,000</td>
</tr>
<tr>
<td>(1 \times 10^{-5})</td>
<td>230,000</td>
<td>460,000</td>
</tr>
<tr>
<td>(1 \times 10^{-6})</td>
<td>2,300,000</td>
<td>4,600,000</td>
</tr>
</tbody>
</table>

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Table II. Approximate minimum numbers of $M_1$ and $M_2$ plants required for the induction and expression of mutation ($u = 1 \times 10^{-4}$) after LD 50 dose. $M_2$ seed harvested from five spikes each representing one treated cell.

<table>
<thead>
<tr>
<th>Number of treated cells</th>
<th>$M_2$ plants per $M_1$ spike</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>$M_1$ seeds treated</td>
<td>25,000</td>
</tr>
<tr>
<td>Fertile $M_1$ plants</td>
<td>10,000</td>
</tr>
<tr>
<td>$M_1$ spikes harvested</td>
<td>5,000</td>
</tr>
<tr>
<td>$M_2$ plants screened</td>
<td>250,000</td>
</tr>
<tr>
<td>Expected mutants</td>
<td>2.5</td>
</tr>
</tbody>
</table>

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INDUCED MUTATIONS FOR IMPROVING MILLETS,
APOMICTIC CROP PLANTS AND VEGETATIVELY
PROPAGATED GRASSES

Glenn W. Burton
USDA, SEA/FR, Coastal Plain Station, Tifton, GA 31794

ABSTRACT

Mutation breeding is a useful tool for the genetic improvement of many crops. It has created early maturing lines of pearl millet (Pennisetum americanum) inbreds Tift 13, Tift 18, and Tift 23 that flower 3 to 4 weeks earlier than the normal lines when planted in the spring. These appeared in the $M_2$ generation in plants from seed treated with ethyl methane sulfonate (EMS) and/or thermal neutrons. To have created early forms of these inbreds by backcrossing from naturally occurring early types would have required more time and effort. By irradiating seeds, downy mildew resistance was induced in Tift 23A used for hybrid pearl millet seed production in India. Recurrent exposure of pearl millet seeds to thermal neutrons and EMS has not increased forage yields but has supplied many simply inherited mutants useful in basic research investigations.

Exposing seed of apomictic dallisgrass, Paspalum dilatatum var. pauciciliatum, to thermal neutrons quadrupled mutation frequency and created mutants in the $M_1$ generation that "bred true" and proved that apomixis had not been broken. None of the mutants surpassed its untreated parent in ergot resistance or yield of forage or seed.

Sugarcane, Saccharum officinarum, is a highly heterozygous, vegetatively propagated grass that has been subjected to mutation breeding. Exposing dormant stem buds to 2 to 6 kR has produced numerous mutations in the $M_1$ generation. Among those reported from India was a red-rot-resistant clone which showed no morphological or yield differences from the original clone.

The Cynodon dactylon x C. transvaalensis turfgrass hybrids (Tifgreen, Tifway, and Tifdwarf) are sterile triploids that are propagated vegetatively. They can only be improved by natural or induced mutation. Exposing dormant rhizomes of these grasses to 7 to 9 kR of gamma rays produced 158 $M_1$ mutants. After several years of evaluation, nine appear to be better than their parents in one or more characteristics.

Mutation breeding is a useful tool for the genetic improvement of many crops. It may be the most efficient method of adding an easily identifiable character such as earliness or dwarfness to an otherwise outstanding variety. In such cases mutation breeding offers two advantages. It usually alters the variety little if any and requires less time than conventional breeding procedures.

Mutations, either spontaneous or induced, offer the only means of improving sterile varieties such as the vegetatively propagated triploid bermudagrass hybrids. Most sterile varieties are highly heterozygous. If so, exposing dormant bud material to gamma rays or some other mutagen can greatly increase mutant frequency over that arising spontaneously. Such exposure can also increase by many fold the breeder's chances of improving the sterile variety.
Variability in germplasm collections resulted from spontaneous mutation. Induced mutations are similar to those arising spontaneously. Breeders using mutation breeding must realize that thousands of years and millions of plants produced the spontaneous mutations in the world. Although mutant frequency can be increased many fold by the proper use of mutagens, the frequency of specific mutants is low and thousands of plants must be screened to discover a specific type. Many of the failures of mutation breeding have been due to the use of populations that were too small.

**Pearl Millet**

Pearl millet, *Pennisetum americanum*, is a robust warm-season annual grass grown primarily for forage in the U.S.A. and for grain in the rest of the world's semiarid tropics. It is a highly cross-pollinated diploid (2n = 14) with a protogynous flowering habit that permits selfing by bagging and hybridizing without emasculation.

In 1962 mutation breeding of pearl millet was begun at Tifton, GA with three main objectives: 1) To create yield gene mutants affecting general and specific combining ability; 2) To provide more information on the number and nature of yield genes; and 3) To supply mutants for basic research and crop improvement. Seed of 10 well-established inbred lines were subjected to three cycles of recurrent treatment with thermal neutrons (TN) and/or ethyl methane sulfonate (EMS) before selecting lines for study. The effect of these treatments on combining ability in pearl millet, studied for 11 years, led to the following conclusions (5, 6, 7): Normal lines, which looked like the controls, selected from lines subjected to three cycles of mutagen treatment, were compared with controls in 3 x 3 or larger Design II hybrid matings in 9 x 9 forage-yield trials. The 1,637 singlecrosses between normal lines from mutagen treatment failed to exceed the 825 control singlecrosses in average forage yield or highest forage yield. These results suggest that pearl millet has many specific-yield genes, none of which exerts a very great effect on yield. Genetic variances estimated from the Design II diallels were not significantly altered by mutagen seed treatment. EMS treatments increased the percentages of nonadditive genetic variance over that in the control and TN treatments, but the failure of any of the 486 hybrids from EMS lines to outyield the best control hybrid suggested that these variance estimates might not be significant. The results of this study suggest that attempts to improve the combining ability of inbred lines of pearl millet by mutagen treatment without several cycles of recurrent selection are not likely to succeed.

This investigation produced many simply inherited mutants that will be useful for basic research and crop improvement. Mutants of outstanding inbreds Tift 13, Tift 18, and Tift 23 that mature 3 to 4 weeks earlier when planted in the spring will be useful in developing early hybrids. The trichomeless gene tr that reduces water loss from leaves up to 35% may help increase drought tolerance and water use efficiency in this crop (3, 11, 12). Other mutants with economic potential include those with genes for resistance to downy mildew, *Sclerospora graminicola*, male sterility, dwarfness and possible apomixis. Mutants for basic research include over 1500 chlorophyll-deficient seedling types (many of which are probably controlled by the same gene), a prostrate type, and many gross morphological variants, translocations, and inversions.

Jain and Pokhiryal irradiated seed of the downy mildew-susceptible Tift 23B and developed the male sterile line 5071A which showed a high degree of field resistance to downy mildew in India (9). By 1975, it had replaced Tift 23A in hybrid pearl millet seed production in much of that country.
Apomorphic Crop Plants

Apomixis may be simply defined as vegetative reproduction through the seed. Apomictic varieties breed true. Their heterosis and all other desirable traits are maintained from one generation to another. Only enough isolation to prevent mechanical mixing is required for the seed increase of apomictic varieties.

A number of perennial grasses are apomictic. Kentucky bluegrass, *Poa pratensis*, is a facultative apomict. It produces both sexual and apomictic plants and the apomictic plants usually produce some sexual offspring. A number of tropical grasses such as *Paspalum dilatatum* and *Panicum maximum* are obligate apomicts and rarely, if ever, produce sexual progeny.

Obligate apomicts cannot be hybridized when used as female parents because they give offspring like themselves. Apomicts can be hybridized when used as males because apomixis affects only the female part of the floret. Thus the genetic improvement of apomictic plants by hybridization requires sexual females. Sexual females can be easily found by selection in a population of a facultative apomict. The lack of a sexual female has prevented the genetic improvement of more than one obligate apomictic species. A method for inducing sexuality in obligate apomicts would be of great value to plant breeders.

Julen reported that treating seeds of an apomictic plant of *Poa pratensis* produced some sexual offspring (10). Doubters have suggested that because *Poa pratensis* is a facultative apomict, Julen's apomictic plant might have produced sexual plants without treatment. A desire to resolve this question and the need for a sexual plant of prostrate dallisgrass motivated the research described below.

Prostrate dallisgrass, *Paspalum dilatatum* var. *pauciciliatum* Parodi is a decumbent perennial that is more persistent, more productive, and more resistant to foliar diseases than the common type. Its 40 chromosomes are extremely irregular at meiosis and its seed set is less than 50%. All evidence indicates that it is an obligate apomict.

To study the effect of radiation on prostrate dallisgrass, seeds were exposed to 15 to 20 hours of thermal neutrons (flux $6.48 \times 10^4$ cm$^{-2}$/sec) and 20 and 30 kR of x-rays, the best of several dosages tested (4). Approximately 1000 seedlings from each of these four treatments and an untreated check were planted in pots in the greenhouse and were later spaced planted on 1.3 m centers in 80-plant-plots replicated 13 times. These plants were grouped into 81 classes based on all combinations of three classes for internode length, leaf length, leaf width, and erectness of growth habit. All plants were examined for evidence of ergot resistance. M$_2$ 15-plant progenies from open-pollinated seed from 259 to 305 M$_1$ plants of each of the five seed treatments were studied. The data collected permitted the following conclusions:

The best radiation treatment, 20 hours of thermal neutrons, compared with the check, increased the frequency of M$_1$ vegetative and floral mutants more than 4-fold and nearly doubled the number of vegetative classes.

The occurrence of mutants in the M$_1$ generation proved that prostrate dallisgrass is heterozygous, a conclusion inferred by earlier cytological studies. If the radiation treatments had induced sexuality, M$_2$ progenies should have been variable. A thorough study of the 15-plant progenies from 1125 M$_1$ plants from irradiated seed revealed that all were uniform. Normal plants had uniformly normal progenies and mutants had uniform...
progenies like their mutant parent. Thus, radiation failed to induce sexuality in the obligate apomictic prostrate dallisgrass. It is significant, however, that the irradiation treatments increased the frequency of mutations in an obligate apomict and that these mutations "bred true." Thus until sexual females can be found, improvement in heterozygous obligate apomicts may be achieved by mutation breeding. Exposing seeds to mutagenic agents should be the most efficient method for inducing mutants in obligate apomicts.

The limited mutation breeding practiced in this study failed to increase forage yield, seed yield, seed quality, or ergot resistance. It did create several interesting mutants including a broad-leafed mutant that was leafier and no doubt produced better quality forage than the normal type. Unfortunately, this mutant was much less vigorous than the normal type and because it and the normal type were obligate apomicts, it was impossible to transfer this leafy character to the normal type of prostrate dallisgrass.

Vegetatively Propagated Grasses

Sugarcane

Sugarcane, Saccharum officinarum, is a vegetatively propagated grass that has been subjected to mutation breeding. It is a high polyploid, is highly heterozygous, and has given mutants in the M₁ generation when stem cuttings have been exposed to gamma rays. In India, Rao et al. (14) irradiated stem cuttings of clone C0449 and induced mutations resistant to red rot disease. Other morphological changes resulting from exposing sugarcane to gamma rays include changes in rind color, stalk size, stunting, glabrous leaf sheath, and resistance to herbicides (8).

Heinz summarized mutation breeding with sugarcane in 1972 (8). Excerpts from his paper follow:

"Many factors influence the use of induced mutations in the improvement of sugar-cane. The size and efficiency of the current sexual breeding program would, to a large degree, determine the amount of effort which might be devoted to using induced mutations. In those countries with a limited or no breeding program, greater weight might be placed on "mutation breeding", especially if a serious disease or other pest were to threaten the industry. This could be important if resistant, high-yielding clones are not available in the country, because most areas have a lengthy quarantine period (up to two years) designed to prevent the introduction of new pests."

"Optimal exposure of sugar-cane buds to gamma rays for high survival and high mutation rate would be between 2 and 6 kR. Since each clone reacts differently, one should work with a range of exposures to obtain the optimal situation for each clone."

"Under certain circumstances, especially where rapid methods of screening are available, irradiation of vegetative cuttings could result in rapid recovery of mutant types. This is especially true when screening for disease resistance, where the inoculum could be applied directly after irradiation. Those plants free of the disease could then be repropagated. The same would apply where easily recognized morphological changes can be observed, such as selection for non-flowering in a heavy-flowering clone."

"The advantages of using cell cultures in the recovery of induced mutations include those mentioned above, plus the ease with which
homologous plants can be recovered. Cell cultures are easy to handle, can be used under most conditions, and lend themselves to the use of chemical mutagens, which would be useful in countries not having irradiation facilities. Large numbers of plants can be produced from cell cultures in a short period of time (three to four months) and can be screened for the desired characteristics within six to eight months after starting a program. One disadvantage is the inability to re-differentiate large numbers of plants from some clones."

The use of induced mutations has a place in the improvement of sugar-cane. The following three examples illustrate where it might be most useful:

(1) In Queensland, Australia, Fijiu disease (virus) has been found after an absence of 18 years. A susceptible clone (NCo 310) was planted on large acreages (80% of the 1970 production) with no resistant, high-yielding alternate clone being immediately available. Research workers have initiated a program to induce mutations for resistance to Fijiu disease in NCo 310.

(2) In the Sudan, the sugar-cane industry is threatened by a serious outbreak of smut disease (Ustilago scitaminea). This country is dependent on clones from an outside source. A program using induced mutants for resistance, until an effective sexual breeding program is established might pay off.

(3) Smut disease poses a threat to the Hawaiian sugar-cane industry, the disease being found for the first time in 1971. Fortunately, it is a serious threat only to one of the less important clones (H49-3533); the two major clones in the industry (H 50-7209 and H 59-3775) have not yet taken infection under natural field conditions. However, several clones showing the potential of outyielding H 50-7209 are highly susceptible. A program of inducing mutations for resistance is being carried out with these promising clones."

Bermudagrass

Wide crosses are usually sterile and must be propagated vegetatively if they are to make an economic impact. Tifgreen, Tifway, and Tifdwarf bermudagrasses for turf and Coastcross-1 bermudagrass for hay and pasture are examples (1, 2). These bermudagrasses, like all wide crosses, are highly heterozygous. Heterozygosity is essential for the appearance of mutants in the M1 generation and the greater the heterozygosity, the greater the frequency of mutants that may be expected from any treatment. If wide crosses are sterile, only M1 mutants can be produced.

All interspecific C. dactylon x C. transvaalensis hybrids are sterile and shed no pollen. This makes them attractive lawn grasses for people who are allergic to bermudagrass pollen. Such sterility facilitates their control, yet it imposes no serious handicap on their use because they can be easily propagated by planting sprigs. The sterility of these hybrids does, however, prevent their improvement by the common plant breeding methods of hybridization and selection.

The success of the natural mutant Tifdwarf suggested several years ago that increasing the natural mutation rate with the aid of mutagenic agents could create other useful varieties. Such mutants should retain most of the superior traits of the "Tif"-bermudagrasses while differing in such traits as plant color, size, and pest resistance.

Dormant rhizomes cut into one- and two-node sections of Tifgreen and Tifdwarf were treated with EMS and gamma rays from Cobalt 60. The
EMS treatments failed, but exposing dormant rhizomes to radiation produced 71 mutants. A gamma dose of 9 kR that reduced shoot emergence to 40% of the controls was used. In the first study 50 of the 71 mutants produced involved a whole propagule (single stolon developing from a treated bud) (13). When increased in 3m² plots, some of these were mixed with other mutant types or sectors. These sectors were evidently present in the original mutants but were too small to be noticed. Once these new variants were separated, increased and established, further changes were minimal.

Two years later, dormant rhizomes of Tifgreen, Tifdwarf, and Tifway exposed to 7 and 9 kR were similarly evaluated and produced an additional 87 mutants (2).

These 158 mutants were increased and planted in plots at Tifton, Ga. and Beltsville, Md. where they have been evaluated for several years (2). Some of the small, slow-growing mutants are much smaller than Tifdwarf and seem to have no value except perhaps for miniature gardens. Other mutants that seemed better than their normal parent early in the test period now appear to be no better, if as good. Nine still seem to be better than the normal checks in one or more characteristics. Two of these mutants were immune to root-knot nematode in a greenhouse test. Two seem to be able to tolerate attacks from several nematode species without loss of vigor. One mutant rarely produces seed heads. These nine mutants and their parents have been planted in 8 x 10 m field plots in triplicate where they will be subjected to several different kinds of management with and without nematode control. Any of these mutants that surpass their parents in this test will probably be named and released.

Coastcross-1 bermudagrass, Cynodon dactylon, is a sterile F₁ hybrid between Coastal bermudagrass and P.I. 255445 from Kenya (1). Although Coastcross-1 bermudagrass yields about the same amount of dry matter as Coastal, cattle consuming Coastcross-1 as hay or pasture have gained 30% faster and have produced as much as 50% more liveweight gain per hectare because it is 12% more digestible. Coastcross-1 is less winter hardy than Coastal and is not a dependable perennial in most of the southern USA.

Coastcross-1, like its Kenya parent, has no rhizomes. Because its Coastal parent had rhizomes, it appears that one or more dominant genes for no rhizomes from the Kenya parent may be masking rhizome development in Coastcross-1. If the dominant gene(s) could be destroyed and Coastcross-1 could be made rhizomatous, its winter hardiness would be increased materially. Radiation of sprigs could destroy the dominant genes or make other changes that would increase the winter hardiness of this grass. Because Coastcross-1 is sterile, it cannot be made more winter hardy by conventional breeding methods.

Radiation breeding of Coastcross-1 was begun June 21, 1971. The main objective was to increase winter hardiness by restoring rhizomes or developing more winter hardy mutants. Some 400,000 freshly cut green stems of Coastcross-1 were packed into 35 × 45 × 90 cm bales with a standard hay baler. These were trucked to the University of Tennessee Atomic Energy Commission Laboratory at Oak Ridge, Tennessee where they were exposed to 7 kR. They were then trucked to the Mountain Experiment Station at Blairsville, Ga. where they were broadcast and disked into the soil and were sprayed with 2,4-D to control weeds. Good establishment resulted and 25 chlorophyll-deficient stem sectors were observed in the fall. Four tiny plants from irradiated material survived -18 C. All plants from non-irradiated stems winter killed. One of these that appeared to develop a few more rhizomes was named Coastcross-3, was increased, and
included in a replicated yield trial with Coastcross-1 and several other bermudagrasses planted in 1974. The yields taken in 1975 and 1976, following mild winters showed no significant difference in these two bermudas (Table 1). In 1977, however, following the moderate 1976-1977 winter, Coastcross-3 had a significantly better spring vigor rating and produced more dry matter than Coastcross-1. Both hybrids were almost completely killed in the severe winter of 1977-1978 as evidenced by the spring vigor rating in Table 1.

Table 1. Dry matter yields of Coastcross-1 and Coastcross-3 (a 7 kR mutant) at Tifton, Ga.

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<tr>
<td>Coastcross-1</td>
<td>8.0</td>
<td>15.2</td>
<td>15.2</td>
<td>6.3</td>
<td>4.4</td>
<td>6.8</td>
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<tr>
<td>Coastcross-3</td>
<td>8.9</td>
<td>16.7</td>
<td>15.7</td>
<td>8.8</td>
<td>3.6</td>
<td>6.6</td>
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<tr>
<td>5% LSD</td>
<td>1.4</td>
<td>1.4</td>
<td>1.7</td>
<td>2.5</td>
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* Spring vigor ratings 1 = no injury; 7 = dead

To increase chances for success, a million or more freshly cut green stems of Coastcross-1 were exposed to 7 kR at Oak Ridge and planted at Blairsville, Ga. for several years beginning in 1972. But a series of mild winters delayed a satisfactory winter kill until 1976-77. In the spring of 1977, we selected six plants that had survived at Blairsville and included them in a replicated yield trial at Tifton. Early results from this trial suggest that these plants are no more rhizomatous than Coastcross-1. They appear to be equal to Coastcross-1 in performance and may yet prove to be more winter hardy.

All bermudagrasses involved in the forage breeding program at Tifton are tetraploids (2n = 36). Recent studies of rhizome development in crosses involving the Kenya bermudas indicate that more than one dominant gene is responsible for the absence of rhizomes. It is possible, therefore, that more than one dominant gene is responsible for the lack of rhizomes in Coastcross-1. It is also possible that the first mutant that produced a few rhizomes may have one less dominant gene for lack of rhizomes. If so, this material would be more apt to respond favorably to mutation breeding. Irradiating about 1 million sprigs from this clone in 1977 produced some chlorophyll-deficient mutants but no rhizomatous mutants.

A severe drought at Blairsville, Ga. in June, 1978 at the only time when the Cobalt 60 source was available at Oak Ridge, Tenn, prevented further attempts to create rhizomatous mutants of Coastcross-1. We recognize, however, the importance of population size in mutation breeding and plan to repeat the 1977 study in 1979.

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ABSTRACT

Data are presented from which it may be concluded that various phenotype patterns can be changed by mutation in numerous gene loci, involving blockings of protein (enzyme) chains as in the chlorophyll and eceriferum mutants. The chlorophyll types are generally inviable under ordinary conditions. The eceriferum mutants, on the other hand, often give a high viability. Other types, such as those changing the photoperiod and resistance patterns, are directly useful in cultivation or may be suitable partners in a continued recombination programme. The macrolepis and hexastichon mutants, caused by changes in one or a few gene loci, often display a rather good viability in spite of the extreme morphological alteration.

INTRODUCTION

At a meeting of an IAEA study group in Buenos Aires (1970) the author presented an analysis of seven phenotype patterns in barley in relation to their genetic backgrounds. These patterns comprised chloroplast and chlorophyll formation, eceriferum and erectoides types, praematurum and intermedium mutations, as well as mutations for mildew resistance. Each pattern had a genetic background consisting of numerous or several genes and alleles with discrete effects. Especially informative in this respect were the eceriferum and chlorophyll mutations. The eceriferum and erectoides types showed a pronounced mutagen specificity.

At the XIVth Genetics Congress in Moscow (1978) the author referred to an article by N.I. VAVILOV (1922) dealing with "homologous series in variation". This concept was at the time built on explorations by VAVILOV and his associates collecting primitive materials in numerous cultivated species. VAVILOV then described the parallel patterns of variation in morphological and physiological respects. As a matter of fact, a student of crop plant diversity may forecast the existence of both useful and useless characters, although unknown at the time, in primitive populations and varieties. Such a polymorphy is, of course, especially evident in the gene centers of the respective species.

By induced variation, applying ionizing radiation or chemical mutagens, a modern mutationist is able to repeat this ancient differentiation and to extend it in highly advanced modern varieties, too. There seems to be no doubt that - with increased information - a new variation may appear which is up to now not found or no longer exists in the primitive centers or is unknown in the international gene banks.

New facts have accumulated in barley concerning the phenotype patterns studied in 1970. We also know a good deal more about the viability and productivity of induced mutations: factor mutations (whatever genetic changes these may comprise) and more or less profound chromosome rearrangements (translocations, inversions, duplications).

Certain rules for the induction of new variation have been formulated. Many characters studied were discussed already by VAVILOV: loss of waxes (eceriferum mutants), immunity or resistance (for instance, to powdery mildew), chlorophyll and chloroplast breakdown, short-awnedness of spikes (breviaristatum mutants), density of spikes (erectoides mutants). In many phenotype patterns of barley numerous gene loci are involved, in chlorophyll mutations several hundred, in eceriferum mutants more than sixty, in resistance to powdery mildew ten to twenty, etc. Since so many genes are generally
involved in individual characters, there is really no question of homologous but rather analogous or parallel series.

In the case mentioned, we have to deal with characteristic morphological or physiological changes. In addition, however, there exist in a diploid crop plant as barley numerous (perhaps innumerable) cases of chromosome breakage leading to chromosome rearrangements without striking or even noticeable morphological changes. Indeed, it is astounding that these gross intragenomonic changes are not generally accompanied by more deviating phenotypes. However, these chromosomal rearrangements possess other consequences of evolutionary significance. They regularly give rise to $F_1$-sterilities when crossed to the original variety and they change the linkage relationships. In many instances, it could be shown (GUSTAFSSON et al. 1967, GUSTAFSSON et al. 1971) that such chromosome rearrangements may be accompanied by small phenotype alterations really acting as a sort of "modifiers" in quantitative characters as, for instance, culm and spike lengths.

In this study, the following characters in barley will be considered:

1. chlorophyll mutations,
2. eceriferum mutations with partial or whole wax inhibition,
3. mildew resistance,
4. mutations leading to photoperiod insensitivity (relating to earliness of heading and maturity).

In the article of the XIVth Genetics Congress, a full discussion of the so-called Peloria character of Linaria vulgaris (toad-flax) was also presented. Instead, some alleged one-gene conditioned phenotype changes as hexastichon and macrolepis (5) will be briefly discussed. The listed characters range from a definite multilocus to a proposed one-locus condition.

(1) Chlorophyll mutations

The breakdown of chlorophyll formation or rather, the control of normal plastid structures - with full pigment synthesis and efficient photosynthesis - is related to a very high number of different genes of the barley chromosomes, in fact, at least two- or three hundred. In addition to the chromosomal genes, there also occur plastid genes (WETTSTEIN et al. 1971, 1973). Multiple allelism is common. A recent analysis (1978) has shown that the enzyme ribulose-1, 5-biphosphate-carboxylase (the most abundant single protein species on earth), can be dissociated into two types of sub-units. In Nicotiana species, one sub-unit (with molecular weight of 55000) is maternally inherited. Another small sub-unit (with a molecular weight of 15000) is inherited in a strict Mendelian fashion. This enzyme participates both in the photosynthetic carbon reduction cycle and in the photorespiratory carbon oxidation cycle. In a previous paper, WETTSTEIN et al. (1971) described the locus multitude in various phenotypical groups of chlorophyll mutation (albina, xantha, viridoalbina, tigrina, zonata, viridis, etc.). The number of loci was respectively: albina - 31 gene loci of 33 examined mutants, xantha - 20 gene loci of 69 mutants, viridoalbina - 14 gene loci of 20 mutants, tigrina and zonata - 16 gene loci of 27 mutants. Among 49 viridis mutants, five gene loci were identified with more than one mutant allele. In Fig. 1, the lesions in chloroplast biogenesis and chlorophyll biosynthesis are indicated as affected by 12
xantha genes and one albina gene. The gene blocks in biogenesis and bio-
synthesis are indicated by arrows.

In a paper of 1954, GUSTAFSSON made a rough calculation of the possible
number of loci for chlorophyll mutation types in barley: 100-125 loci for
albina mutants, 100-125 loci for viridis mutants, 10-15 loci respectively for
xantha and albiviridis types. The remaining rare types of mutants might
according to this primitive method of calculation approximately comprise 15-20
loci. Probably, the total number of gene loci for chloroplast and pigment
breakdown is even higher.

The chlorophyll mutations are, in general, lethal as homozigotes. Some
viridis mutants are viable. In heterozygous condition, some lethal chlorophyll
mutations may increase viability and productivity.

(2) Eceriferum mutants

The genetical analysis of this most interesting type of barley mutation
has been carried out by U. LUNDQVIST and D. von WEITSTEIN, the physiological
and biochemical analysis by P. von WEITSTEIN-KNOWLES and her co-workers
and yield analysis by the present author. The isolated induced and spon-
taneous eceriferum mutants now reach a number of 1400. Of these, 1202 cases
have been localized to 69 loci. Five dominant mutant loci have been found.
It can generally be said that, although recessive mutants are in the majority
for most mutant types, dominant mutations will also occur. Up to now seventeen
mutants have been localized to six of the seven barley chromosomes (SOGAAED,
v. WEITSTEIN-KNOWLES 1979). None has so far been referred to chromosome 6 but
the number of localized genes is still too low to permit definite conclusions
on this point. The previously mentioned mutagen specificity has again been
verified (LUNDQVIST unpubl.), especially with regard to locus cer-i, which
abundantly mutates with neutrons, less so with X-rays and scarcely with chemi-
cal mutagens. This is in striking contrast to locus cer-j, which has up to
now given no mutant case in neutron treatments, a reasonable number with X-rays
and an abundant number with chemical mutagens.

There also are other striking specificities. The three genes: cer-a,
cer-q and cer-u, lie close to one another on chromosome 4 (SOGAAED 1973).
According to LUNDQVIST (unpubl.) and WEITSTEIN-KNOWLES (1979) in eleven out
of 25 mutant cases (= 44%) two or three of these genes have mutated together
after neutron treatment; only one of 19 cases (= 5%) with X-rays and also once
of totally 426 cases (= 0.2%) with chemical treatments. The induced rate of
eceriferum mutants is much higher with chemicals than with neutrons and X-rays.
On the other hand, double or triple cer-a mutants are most easily achieved by
ionizing radiation, especially neutrons.

The wax constitution of barley as well as of other plant species is
complex. The waxes consist of primary alcohols, free fatty acids, \( \delta \)-diketones,
hydroxy-\( \delta \)-diketones, aldehydes, esters, hydrocarbons. There is a definite
organ specificity with regard to the occurrence and amount of different wax
constituents. There even are differences in wax constituents between spikes
and their awns, as for instance, in the mutant cer\textsuperscript{271}, where the awns, in contrast to the spikes, are lacking in $\beta$-diketones and aldehydes.

The mode of origin of different waxes has been diagrammed by WETTSTEIN-KNOWLES (Fig. 2). A special elongation complex successively adds "$C_2$-units" to $C_{16}$ and $C_{18}$ fatty acids reaching full lengths of $C_{36}$ acids or higher. The fatty acids are then worked upon along a "reduction pathway" leading to esters, primary alcohols and aldehydes, an "elongation complex", just mentioned, giving rise to fatty acids of various lengths and, then, a "decarboxylative pathway" leading to hydrocarbons also with various carbon numbers.

WETTSTEIN-KNOWLES (1976) indicated the existence of blocks for eight different eceriferum mutants affecting the epicuticular spike waxes (Fig. 3).

The numerous eceriferum gene loci, with their organ specificities, indicate analogous end effects but no true homology of general gene or enzyme action.

(3) Mildew resistance

Powdery mildew is a common disease in European cultivars of barley. The genetic situation is quite complex but differs from what is valid for chlorophyll and eceriferum mutants. In the eceriferum group a very high number of individual genes exist, apparently well distributed over the barley genome (the possible exception of chromosome 6 is not yet verified). The same seems also to be true of the chlorophyll mutations.

In the case of powdery mildew a rather restricted number of genes for resistance have been localized to chromosomes 4 and 5, more gene loci known for chromosome 5 than for chromosome 4. The individual gene loci, especially of chromosome 5, seem to be large clusters of alleles with differences in resistance patterns.

The gene loci in chromosome 5 are depicted in Figure 4. The order of location is $ML$-at, $ML$-a, $ML$-k, $ML$-d, $ML$-nn, $ML$-p. Five loci contain dominant resistance alleles. One gene, $ML$-d, is recessive. In the gene-rich chromosome 5 also the eceriferum loci cer-e, cer-ai and cer-af are located, as are also the erectoides locus ort-b and the matura locus mat-a (= ea-k; the Mari gene for earliness and photoperiod insensitivity, $v$, below).

On chromosome 4 two loci for mildew resistance are known for certain. One is the dominant $ML$-g. The numerous alleles of this locus are said to condition resistance to a limited number of mildew races. Most interesting is the behaviour of the locus $ML$-g, with its recessive character, especially studied by WIBERG, JORGENSEN and JENSEN. Special reference is made to the publication of JORGENSEN (1977). In chromosome 4 also six eceriferum loci (HAUS 1978) are known (cer-i, cer-zh, cer-zg and the three remarkable loci cer-o, cer-o and cer-u, as well as a gene for the six-row character $v$-g).

Some mutated alleles for $ML$-g resistance seem to be promising sources for further plant breeding efforts.
The contrast in architecture of eceriferum and mildew resistance genes seems to be evident. Apparently, a very high number of genes, distributed over the genome, is characteristic of the wax conditioning genes, with their organ, compound and mutagen specificities. The mildew resistance genes are concentrated to two chromosomes with a limited number of gene loci but with a high number of clustered alleles, having partially different modes of reaction.

In the years 1967 to 1976, a series of mutagen treatments were carried out in order to screen for mildew resistant mutations using definite races of infection. Among 176 400 tested plants 57 mutants were detected, 44 of which have been closely analysed. Fourteen mutations comprised the ml-o locus. These mutations have occurred in the Svalby materials of Poma, Mari, Kristina and Pallas barley. The rate of the ml-o-mutants reaches 23% of all enumerated cases of resistance and they arise once in 12 700 tested plant progenies. Some ml-o-mutants are high-productive also in direct comparative trials.

4 Photoperiod insensitivity

In a meeting in Vienna on "Induced mutations in cross-breeding" (1976), GUSTAFSSON and LANDQVIST reported that common greenhouse testings, involving short-day conditions of 8 hours of day light and 16 hours of artificial darkening, still permitted genotypes possessing the Mari-gene (mat-a) to produce fertile spikes and well-developed seed. These simple experiments were repeated in 1977 and 1978 with similar results. In the year of 1978 ten Mari replicates gave an average heading time of 69.3 days. Its cross variety Mona, also in ten replicates, gave a corresponding heading time of 69.8 days. The parent variety Bonus seemed to be able to form spikelet rudiments but these did not elongate and Bonus never headed, nor formed seeds.

A series of mildew resistant mutants from Mari barley gave heading times between 70.1 and 75.0 days after sowing. Mutations in the matura-loci b, c and f (mat-b7, mat-b13, mat-c16, mat-c15, mat-f21) did not head. They evidently possess another physiological background for early heading under ordinary long-day field conditions. It ought to be noticed here that the matura-a mutants, as indicated for the allelic mutant early-k (ea-k, TAKAHASHI and YASUDA 1971), turn yellowish-green under extreme short-day conditions.

However, another non-allelic gene, mat-e18, regularly heads under short-day conditions, although the heading time is somewhat delayed: in 1977 it was 88.0 days and in 1978 79.1 days, distinctly later than the various mat-a types. The mat-e mutant is normal-green and deviates in this respect from all certain cases of mat-a mutants. There are some instances of delayed short-day heading in some other normally green mutants (matura 742, 743 and 745), with some individuals actually heading, some with flag-leaves and some not entering the heading phase. Whether these represent
the _e_-locus or some other locus or loci, is not yet known. Apparently, there exist differences also in short-day reaction between individual genes or alleles.

We may safely conclude that the _mat_-loci _a_ and _e_ possess different degrees of short-day adaptation. The _matura_- _a_ are generally quite pronounced in this respect and adapted for short- as well as long-day conditions but less well adapted to high thermoperiods (high temperatures), where they show a curtailed heading with a definite loss of productivity. Nevertheless, the _matura_- _a_ alleles are outstanding in their wide climatic adaptability, combined with a generally high production capacity, when summer temperature is not too high. This fact was expressed by SIGURBJÖRNSSON (1975-1976) in the following way:

"The mutation characterizing Mari gave it remarkable earliness and hardiness so that two-row barley broke through a climatic barrier thereby extending its area to the north. During a period of unfavorable summers in Iceland from 1964-1971, Mari was the only barley variety which consistently produced an economic crop while all other varieties were wiped out. Icelandic farmers call it "hurricane proof". Due to its photoperiod insensitivity it can be grown also far to the south including Colombia where Mari and its daughter variety Mona are valuable as breeding material and Mona gives high yields, even under tropical conditions at 350 meters elevation". In its region of origin (the district of Scania in Sweden) Mona was for a long time the widest cultivated barley variety, owing to its high yield, early ripening, good straw and (original) mildew resistance.

That a newly arisen mutation can replace its parent variety also in regions where this is at its best is shown also by the mutant variety Pallas which was widely cultivated in Western Europe for a series of years. In Denmark where Bonus - the parent - was cultivated for a long time, it was successively replaced by Pallas. There are, I consider, definite examples opposing the prevailing views of a general negative correlation between degree of phenotypical differences and viability (yielding ability (v. for instance, STEBBINS 1974)). Viability is certainly correlated with the phenotype character, but also with the genes and alleles acting, and the general phenotype background.

There are a number of different aspects on the phenotypical realization of a character as earliness. The study of induced mutations may help to clarify them: (1) Long-day adaptation with different gradations of earliness, genetically determined, in response to the acting day-length. This also involves genetically determined gradations of earliness in one and the same long-day condition (for instance mutations in barley genes _mat_- _b_ and _mat_- _c_). (2) Short-day adaptation similar as in case (1) with regard to response to a varying day-length or with gradations existing in one and the same short-day condition. (3) Photoperiod insensitivity, i.e., the mutants (or varieties) develop and yield well under highly variable photoperiod conditions (as in the _matura_- _a_ mutants). Also here differences in insensitivity exist, between
different genes (mat-a and mat-e) or between alleles in the individual loci
(as also indicated by different mature-a alleles).

In all instances, also the thermoperiod conditions, or the absolute
levels of temperature, interact with different photoperiod conditions.

(5) The macrolepis character

In 1940, GUSTAFSSON and ÅBERG described a mutant in barley denoted as
"lemma-like glumes", because the outer glumes were enlarged and awned, so
that each spikelet of the mutant showed three long awns. Later on a series
of such mutants were found in Swedish and German experiments. NYBOM (1954)
recorded eight cases. All cases studied were allelic with one another and
also with a spontaneous variant "Triple Bearded Mariout". They differed,
however, from one another both with regard to the size of the enlarged glumes
and the length of the awns. Further mutants were described by NOTZEL (1952)
and STUBBE and BANDLOW (1947), with one mutant denoted as "megalepis".

Some confusion with regard to the locus denotation exists (e, log, y, ex,
lep: NILAN 1964, GUSTAFSSON et al. 1969). The locus "e" is in chromosome 2,
close to the centromere (TSUCHIYA 1973). In the Swedish and German experiments
always one gene locus was involved. NILAN (1964, p. 121) reported findings
that indicated a two-factor inheritance. The second gene e2 or gh, with
fine-awned glumes, was said to lie on chromosome 1 (NILAN i.e., p. 86).

GUSTAFSSON et al. (1969) reported 28 mutants, 22 of which were at that
time allelic, the others not yet examined. Unpublished data show that all
macrolepis mutants isolated are recessive and belong to one and the same
locus, independent of the original parent variety. The expression of the
character varies considerably.

Previous studies indicated that the six-row character, in contrast to
the intermedium type, always is associated with changes in one special gene
locus. GUSTAFSSON et al. (1969) reported 24 cases, 19 of which were inter-
crossed. All belonged to one locus denoted as hex-v. In contrast, the
intermedium types (37 cases in 1964) at that time belonged to 6 different
loci (int-a to int-f). The six-row locus is in the international literature
denoted as v, a gene which lies in the long arm of chromosome 2. One inter-
medium factor is said to lie in chromosome 4. Apparently, the situation is
complex in the case of the intermediate mutants.

There are some indications that the six-row character is also less clear
than hitherto reported from mutation experiments. It appears as if the six-row
character could also be realized in crossings involving different intermedium
loci. Studies are on way to clarify this situation.

The macrolepis and the six-row characters indicate a fairly simple type
of inheritance, which turns out to be more complex already in the case of
intermedium mutants.
The macrolepis character has arisen in all carefully studied strains of cultivated barley. It has also, as mentioned before, been obtained as a spontaneous variant (Triple Bearded Mariout) and has been collected in primitive sources ("ischnaterum", GUSTAFSSON and ÅBERG 1940). A case of parallel variation is evident.

**Conclusions**

In this analysis a series of data have been presented concerning the genetics and biochemistry of some phenotype patterns in barley. Two character patterns - chlorophyll and eceriferum mutants - are conditioned by a great number of genes and alleles, spread over the barley genome. In the case of the chlorophyll mutations there certainly exist no less than 200-300 chromosome gene loci where homozygous mutations lead to increased or complete lethality. Some mutations may, however, increase viability in the heterozygous state. There also exist plastid DNA genes which act on subunits of the enzyme ribulose-1,5-biphosphate-carboxylase.

From a viability point of view the eceriferum mutants react in another way. They concern the wax formation of culms, leaves, spikes, awns. Up to now almost 70 different cer-genes have been identified. The mutated genes inhibit wax formation in various ways. However, a great number of such block mutations - in spite of their biochemical effects - are fully viable with a grain yield per plant or hectare equal to that of the parent line. Some yield data were presented in the paper by GUSTAFSSON (1963). Of the gene loci discussed in this paper cer-i and cer-j, which show different mutagen specificities (p.4), give mutant yields often approaching 100 per cent. The genes cer-o, g and u, closely linked on chromosome 5 and with a specific response to neutron irradiation, also may give a full or almost full mutant viability. Unpublished data indicate the high yield performance of several other eceriferum loci, although differences exist between loci and alleles. The organ specificity, as well as the mutagen specificity, is pronounced.

In the case of a third phenotype pattern - mildew resistance - the number of gene loci is rather restricted. Approximately ten to fifteen genes lie in chromosome 5 and three to four in chromosome 4. Numerous alleles, possessing specificities in race resistance, have been detected in several gene loci. Dominant and recessive mutations were found, as was the case also in other mutant characters (erectoides, eceriferum, intermedium). Especially interesting are the recessive mildew mutants belonging to the locus ml-o. Published data indicate the high yielding ability of several ml-o mutants, some alleles or which seem to be promising sources of resistance in gene recombination work.

"Earliness", i.e., early heading and early maturity, is a complex property. It is in some cases accompanied by features of photoperiod insensitivity (or neutrality) as in the matura-a and, in a way, also the matura-e mutants. Different mutants (alleles) of the matura-a locus are high-yielding, for
instance the mat-a homzygous (Mari), and have shown themselves valuable in world-wide recombination work. The thermpereiod influence has to be con-
sidered. Early mutations, which are still long-day adapted, also occur in
Scandinavian strains (mat-b, mat-c, etc.), although differences in their photo-
and thermoperiod behaviour are evident. No planned selection for increased
long-day reaction has been carried out in Scandinavian mutant materials. But
it is interesting that the original bushy and highly waxy mutant taking part
in the northern Gunilla barley (GUSTAFSSON et al. 1971) was considered to be
especially well adapted to North-Swedish conditions (K. WIKLUND), although
induced in a southern variety (GULL barley).

The high-yielding capacity of many photoperiod insensitive and early
mutants may once more be stressed here (in addition to mat-a also mat-b and
mat-e, etc.). These are of a rather drastic type. In addition to them,
numerous mutations occur, which are of the "modifier type" and also rather
high-yielding.

Finally, under the heading of the macrolepis character some types are
listed, which represent mutations only in one or a few gene loci, although
many alleles with different expressions of the mutated character(s) may
exist. In some cases as the hexastichon and the intermedium types the situ-
uation might be more complex than hitherto supposed. Also, some of these
mutants are quite good yielders. NILAN (!...£.» P« 122) refers to a case of
increased yield in a spontaneous macrolepis mutant.

It must here be emphasised that the phenotype expression of mutants
(induced or spontaneous) may range from rather dramatic to scarcely noticeable.
Both extremes often show a high viability. Mutations with major as well as
minor effects have continually contributed to the process of domestication.
The marked alterations in cultivar morphology are not only sum effects of
added small changes. They also are the result of mutations with discrete
major effects. Around such major changes recombination of genes and further
mutations are continually at work, fitting large and slight changes together
into a balanced whole, where numerous genes come to influence the same pheno-
typic character. This is evident for all sorts of mutant characters, whether
morphological, physiological or biochemical ones, dealing with adaptive
features, resistance patterns, stature, yielding ability, proteins, amino acids,
or alcaloid metabolites.

The genetics of phenotypic architecture is a fascinating theme, which
begins to be opened up.


Figure explanations (Ake Gustafsson: Analysis of Phenotype patterns):

Fig. 1 Diagram of the lesions in chloroplast biogenesis (left) and chlorophyll biosynthesis (right) caused by mutations in 12 xantha genes and one albina gene. (After D. von Wettstein et al., 1971).
Fig. 2 Diagram of biosynthesis of various epicuticular waxes found on barley leaves. The elongation complex successively adds C₂-units to palmitic acid to form elongated fatty precursors. These may enter the reductive pathway, the decarboxylative pathway or be dissociated from the complex. (After P. von Wettstein-Knowles, 1974).

Fig. 3 Biosynthetic relationships of the β-diketones, hydroxy-β-diketones, alkan-1-ol containing esters and alkan-2-ol esters in epicuticular waxes of normal barley and eceriferum (cer) mutants in eight gene loci. After P. von Wettstein-Knowles, 1976.)
<table>
<thead>
<tr>
<th>Locus positions</th>
<th>Locus designations</th>
<th>Interloci distances</th>
</tr>
</thead>
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<tr>
<td>105.3 ± 4.8</td>
<td>Ml-at</td>
<td>23.4 ± 2.9</td>
</tr>
<tr>
<td>81.9 ± 4.0</td>
<td>Pa4</td>
<td>14.5 ± 0.9</td>
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<tr>
<td>67.4 ± 4.0</td>
<td>Ml-a</td>
<td>6.2 ± 1.3</td>
</tr>
<tr>
<td>61.2 ± 4.2</td>
<td>Yr4</td>
<td>1.4 ± 1.4</td>
</tr>
<tr>
<td>59.9 ± 3.9</td>
<td>Ml-k</td>
<td>11.9 ± 2.0</td>
</tr>
<tr>
<td>48.0 ± 4.2</td>
<td>ml-d</td>
<td>4.1 ± 2.9</td>
</tr>
<tr>
<td>43.9 ± 4.3</td>
<td>Ml-nn</td>
<td>13.8 ± 3.3</td>
</tr>
<tr>
<td>30.1 ± 4.3</td>
<td>Ml-p</td>
<td>13.9 ± 5.3</td>
</tr>
<tr>
<td>16.1 ± 3.0</td>
<td>cer-si</td>
<td>1.7 ± 5.7</td>
</tr>
<tr>
<td>14.4 ± 4.8</td>
<td>fs2</td>
<td>3.4 ± 4.0</td>
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<tr>
<td>11.0 ± 5.1</td>
<td>cer-e</td>
<td>0.8 ± 3.1</td>
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<tr>
<td>10.4 ± 4.4</td>
<td>ert-b</td>
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<tr>
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<tr>
<td>-85.5 ± 4.2</td>
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</table>

Fig. 4 The barley chromosome linkage map. Distances are given in centimorgans. The order and positions of some Ml gene loci may not be definite. The position of the centromere is probably slightly above the gene fs2. (After JENSEN, 1978).
ABSTRACT

A plant breeder may utilize the genetic variability from available natural resources, he may build up variability through hybridization, he can induce variability through mutagen treatments or he may use a combination of any of the three for the improvement of crop plants. A number of improved varieties of rice have been developed through mutation breeding. It is shown, how a breeder may utilize mutation induction to achieve successfully his breeding objectives.

INTRODUCTION

Genetic variability is the basic requirement for any crop improvement programme. The sources of this variability can vary. The present day plant breeder may utilize the variability from the available natural resources, he may build-up the variability through hybridization, he can induce variability through mutagen treatments (mutation breeding) or he may use a combination of all three for the improvement of crop plants.

It is now a well established fact that both ionizing radiations and chemicals are efficient in inducing genetic variability in practically all the characters of a crop plant. Success depends upon the character upon which improvement is sought, frequency of mutation that may occur for the trait, the ease of detection of the changed character from the population and also to a great extent on the careful conducted tests from $M_3$ onwards.

The success of a particular programme also depends, may be to a very large extent, in the selection of parent variety, which should be, in general, the best local adaptive variety; in the selection of mutagen doses, the plant population in $M_1$ and $M_2$ and the effectiveness of the screening technique.

In this presentation I shall not attempt to review the work done in rice crop improvement with induced mutations by many scientists, as such reviews are available. Those who are interested may refer to Nayar (1965), Gustafsson and Gadd (1966), Gregory (1972) and Micke et al. (1972). Moreover, since the starting of the FAO/IAEA Co-ordinated programme of research on the use of induced mutations in rice breeding in 1964, FAO/IAEA has published in book-form the research reports of the scientists who took part in the co-ordinated programme (IAEA 1968, 1970, 1971). It is worth mentioning here that much of research that has been carried out with rice and the results achieved are due to the above programme as it lead to more systematic breeding work for achieving definite objectives of economic relevance.

It may be observed from the above mentioned review papers, summaries and research reports that much has been achieved. A number of improved varieties of rice have been developed through induced mutation breeding and mutants have contributed in achieving high yield, short stature and lodging resistance, early maturity, increased protein content, improved grain quality etc.

As a plant breeder I shall try outline in this presentation how a breeder should approach his problem to achieve success in mutation breeding work and I will present the methods used by successful mutation breeders to achieve their objectives.

Choice of Mutagen and Determination of Dose for Favourable Mutations

For inducing genetic variability both physical and chemical mutagens are effective, and there appears no distinct difference in value between mutations induced by ionizing radiations and those induced by chemical mutagens.

* FAO, Plant Production and Protection Division, Rome, Italy since 1979.
Mutation frequency studies provide some basis for estimation of the effectiveness of mutagens at a particular dose. In barley where extensive studies have been conducted mutagen effectiveness was determined on the basis of chlorophyll mutation frequency. The highest mutation frequency achieved through a mutagen treatment could be taken as a measure for comparison of mutagen efficiency. The highest frequency of chlorophyll mutations per 100 M₁ spikes by irradiation with X-rays and neutrons, after treatment of seeds, did not exceed 20 (Kawai, 1969). From experiments with chemical mutagens Kawai concluded that EMS is the most efficient mutagen where the frequency of chlorophyll mutations obtained were 57 per 100 Mₛ spike (conc. 0.6%, time 24 hrs.) Kawai further pointed out that too strong a treatment inducing 50 chlorophyll mutations per 100 Mₛ spike may not be favourable for breeding purpose. This assumption he based on results of experiments with rice where morphological mutant lines (including early heading mutant lines) were tested for yielding capacity. The results indicated that the proportions of mutant lines with a yielding capacity equivalent to or higher than the original variety was lowest where treatment had induced chlorophyll mutations at the highest rate. He concluded that from practical breeding point of view one should not try to produce a large number of overall mutations (relative to Mₛ injuries) but to obtain a higher proportion of mutation of practical use. As an example, he mentioned that the number of mutations possessing a yielding capacity equal to or higher than the original variety per Mₛ spike progeny was highest where treatment with EI (Ethylene Imine) had induced chlorophyll mutations at a rate of 21.1 per 100 Mₛ spikes.

Ehrenberg et al. (1965, cited from Kawai 1969) made analyses of induced variations of grain weight per row and other characteristics in barley and came to similar conclusions. The EI treatment which induced chlorophyll mutations at rates of 7-16% per spike progeny was more efficient in inducing heritable variations of quantitative characters than was EMS treatment, which induced chlorophyll mutations at rates of 12-20% per spike progeny. These results and those of other studies on recurrent irradiations suggest the existence of an optimum frequency of mutations for breeding purpose, as has been suggested by Hänseel (1966, 1967 cited from Kawai 1969).

As our objective is to induce a maximum of favourable mutants we must be careful in choosing the optimal treatment dose of a mutagen for a successful programme.

Plant Population

The size of the plant population in both Mₛ- and Mₛ+ generations is very important in mutation breeding work. For recovery of a desirable mutant a large population is generally required. It is recommendable to have an advance estimate of the Mₛ population needed. There are two considerations involved: An increase in the number of Mₛ lines, which come from a single Mₛ spike or plant, will increase the chance of recovery of a line segregating for a desirable mutant. Again with the increase of number of plants per line the number of mutants which appear in a line increases. Thus, both the increase of number of lines and number of plants per line are desirable to increase the probability of obtaining the desirable mutant. But in practice the Mₛ population size will be limited due to facilities of land, labour etc.

Yoshida (1962) studied the efficiency of selection of an easily detectable mutant from Mₛ population derived from seeds treated with mutagens. According to him, the one-plant-one-grain method (method C) is the most efficient. He also recommended one-plant-two-grain method (method D) and the one-plant-three-grain method (method E) in order to keep both the number of Mₛ- and Mₛ+ plants to a minimum.

Gustafsson and Gadd (1966) discussed the paper of Yoshida and using the denotations of Yoshida and the formula advanced by Freisleben and Lein (1943, cited from Gustafsson and Gadd 1966) calculated the mutation frequencies of the five methods and expressed that around three kernels per spike-progeny (method E) would be the least expensive method in radiation breeding (for details please consult Gustafsson and Gadd 1966).

Brock (1970) also estimated the Mₛ family size for different segregation ratios and levels of probability of occurrence of the homozygote mutant and stated that with an induced mutation frequency of 1/1000 and accepting 90%
probability of occurrence a $M_1$ population of 5000 plants (or spikes) yielding 2500 fertile progenies would be sufficient. The mutant would have to be detected in a $M_2$ population of approximately 50,000 (2,500 x 20) plants. But if the mutation frequency is 1/100,000, the $M_1$ and $M_2$ population would be 250,000 and 5,000,000. Gregory (1973) also gave similar estimates. He stated that for a desired change, say earliness, with a 95% level of probability and with a frequency of 1:1000, even with highly efficient 'einkorn' method approximately 20,000 $M_1$ plants would be required. He further stated that if one is searching for the one out of 10 of the recovered mutants, which yields at least equal to the original variety, approximately 200,000 $M_2$ plants would be required and if one wants to combine earliness, yield and high level of resistance, with an induced expectancy of 1:100,000 then he will need a $M_1$ population of at least 20 billion plants (Yoshida, 1962; Kawai and Sato, 1969).

These figures show the importance of population needed for detection of induced changes and how the whole programme may be complicated if one desires to combine several characters in a single mutant. Success in mutation breeding will therefore greatly depend on the size of $M_1$ population and on the number and composition of characters over which improvements are sought. It is desirable to keep the number of characters at a minimum, preferably at one, and the character should be easily detectable.

Breeding Objectives and Handling of Materials

1. Yielding ability

Increase in yield per unit area of a crop variety is the ultimate objective in most plant breeding programmes. In this respect the variety plays the most important role. Applications of fertilizers, better management, control of diseases and pests etc. may lead to higher production of a variety up to a certain limit but these can not increase the yield spectacularly unless the variety has the potential. As such, to achieve higher production, the variety needs to be improved genetically.

Yield mutants do occur as frequently as other characters after mutagen treatment, but yield being a typical quantitative character, its expression is highly influenced by environmental factors and thus yield mutants are very difficult to identify, particularly on a single plant basis. As a result, the selection procedures for screening of yield mutants, may not be the same as for other, easily detectable characters.

Aastveit (1970) has mentioned two methods for selection of yield mutants. The first one is an indirect method in which case the selection is confined to an easily detectable character which may have a positive pleiotropic effect on yield. After establishment of lines on this selection basis, these lines are to be tested in subsequent generations for yielding ability. Characters such as short-culm, erect habit, stiff straw may be considered in case of rice. Such characters are associated with higher yielding ability in some cases, at least under heavy nitrogen fertilisation. To me it appears to be the prevailing best method for selection of yield mutants for development of high yielding rice strains in developing countries.

The rice varieties that are in general cultivation in the developing countries are all indica type. They are susceptible to lodging, are inefficient users of nitrogenous fertilizers and low yielding. To make these varieties comparable in yield with, e.g. IRRI varieties, a yield increase of 50-100% is needed and this is rather impossible to achieve by keeping the existing plant types. From my own experience with local varieties both in Bangladesh and Burma it may be said that we were unable to detect any high yielding mutant of the above magnitude from any of the varieties along with the existing plant types. The two high yielding mutants (Haq et al 1971) that were identified from a local early maturing variety DULAR were very short in plant height (70 cm against 134 cm of the mother) and the yield was more than double (mother - 1763 lbs/ac, Mutants - 3919, 4599 lbs/ac), the yield increase was due to the increase in the number of tillers (mother - 15.9, mutants 37.4 and 25.0). But unfortunately, later it was observed that both the mutant strains were highly susceptible to bacterial leaf blight and thus could not be released as varieties.
Ganashan (1971) was also able to develop a high yielding mutant strain from the local H$_4$ variety. The yield was about 90% more than the mother (H$_4$ - 61.1, mutant MI-273 (m), 106.4 bushels per acre). In this case also the plant height was fairly reduced (mother 111.70 cm, mutant - 57.6 cm). Incidentally, the yield of this mutant strain compared very favourably with the high yielding introduced variety IR8. The main yield contributing factor was the increase in panicle numbers (av. 15.20 against 11.50).

The other selection method is direct and the selection procedures start with the progeny testing of individual plants similar to the procedures followed in any hybridization programme. The methodology as discussed by Aastveit (1970) for selection of such yield mutants may be followed.

The development of mutant strain MI-273 (m) from the variety H$_4$ is outlined below (Ganashan 1971):

Variety - H$_4$ (a local variety famous for yield in Sri Lanka, av. ht - about 112 cm).

Mutagen - gamma rays (10, 20, 35, 60 kR)
- neutrons (300, 600, 900, 1200, 1600 R)

$M_1$ - The first formed three panicles of each $M_1$ plant were bagged at flowering and each panicle was harvested separately.
- 1414 $M_1$ plant progenies (604 - gamma rays, 810 neutrons) were carried over to $M_2$.

$M_2$ - The $M_2$ generation was raised as ear-to-row progenies - 25 plants per line.
- (I) short culm and dwarf mutants, resistant to lodging were selected and harvested on individual plant basis.
- (II) normal-looking plants in mutated lines harvested as bulk.
- (III) $M_2$ lines which showed no phenotypically detectable mutation were also harvested as bulk.

$M_3$ - group (I) selected plants were grown as plant progenies.
- group (II) and group (III) were grown as bulk.
- only short-culm and dwarf mutants that had more than 50% spikelet fertility and were resistant to diseases were selected.

$M_4$ - The selected mutants were grown in larger plots for further selection.
- Selections based on high spikelet fertility with relatively compact tillering, erect and darker-green leaves, thicker grain size and resistance to diseases.

$M_5$ - Some of the promising mutants (10) were tested for yield and mutant MI-273 (m) originating from 35 kR gamma irradiation recorded the highest yield.

$M_6$ - Superior lines of MI - 273 (m) which were uniform were bulked and included in the Co-ordinated Rice Varietal Trial.
- Programme conducted in different agroclimatic zones along with two other promising varieties, IR8 and Bg-11-11 in the island and two such trials were conducted.

The data showed that mutant strain MI - 273 (m) excelled its parent H$_4$ in yield at all locations. It also outyielded Bg 11-11 in almost all locations and the over all performance of the mutant strain compared favourably with IR8. It was proposed to recommend this strain for mass production in both Maha and Yala seasons.

2. Culm length and straw stiffness

Culm length is another important breeding objective particularly with indica varieties as it is associated with lodging. Although, lodging resistance and short culm length are not always associated, in general the
short culm length reduces the leaning and bending of the culm which is the common cause of lodging. There is evidence that short-culm mutants are fairly easy to achieve through mutagen treatment and these types of mutants can be visually identified. Development of short-culm strains with high yielding ability has already been discussed under high yielding ability.

While short-culm is a desirable trait, under certain high rainfall conditions short-culm varieties may not be suited, as during the growing season, occasional heavy rainfall may cause temporary water logging in the rice fields and the short-culm varieties are submerged and may be destroyed due to such submersion. Such a case was observed in Burma with high yielding introduced variety IR5. This variety was well adapted to Burmese climatic conditions but its cultivation was limited due to its comparative short-culm length. The farmers wanted a variety like IR5 in yielding ability with 10-15 cm. higher plant height. Accordingly, a programme was initiated in 1971 under an IAEA Project and a variety SHWE WAR TUN was released by 1975. The variety was not only higher in culm length but has high grain quality (slender and translucent) and fairly high milling out-turn as compared to mother IR5.

The method of development of SHWE WAR TUN is described below:

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1971</td>
<td>- irradiation of pure seeds - 25 kR, 30 kR, 35 kR gamma rays - 300, 600, 900 rads fast neutrons. &lt;br&gt; - 3000 single spaced plants were grown at ART (wet season) from each dose. &lt;br&gt; - 3000 main panicles were harvested from each dose except 300 Nf. &lt;br&gt; - research harvested in bulk dose-wise.</td>
</tr>
<tr>
<td>1971-72</td>
<td>- on the basis of laboratory examination 468 panicles from all the doses were finally selected. &lt;br&gt; - 468 spike progeny rows - 39 plants per row - were sown at the Hmawbi Farm. &lt;br&gt; - 12,000 single plants from the bulk of 35 kR were also grown. &lt;br&gt; - selected 16 single plants from 3 spikes rows of 25 kR. &lt;br&gt; - selected 10 single plants from 2 spikes rows of 30 kR. &lt;br&gt; - selected 39 single plants from the bulk of 35 kR. &lt;br&gt; The selection was based on grain quality (fine, translucent grains) and plant height (slightly taller).</td>
</tr>
<tr>
<td>1972 (wet season)</td>
<td>- the 65 selected single plants were grown at ART Farm as plant progenies with - 3 rows of 30 plants each. &lt;br&gt; - a bulk population (¼ acre) from 35 kR was also grown for further selection of single plants. &lt;br&gt; - selected 3 progenies out of 16 (25 kR) &lt;br&gt; - selected 6 &quot; 10 (30 kR) &lt;br&gt; - selected 6 &quot; 39 (35 kR bulk) &lt;br&gt; - selected 16 plants (translucent and fine grain) from 35 kR bulk. &lt;br&gt; - selected 3 plants (coloured grain) from 35 kR bulk.</td>
</tr>
<tr>
<td>1972-73 (dry season)</td>
<td>- nine (3 from 25 kR, 6 from 30 kR) uniform lines along with 3 checks (IR5, MA 68-1, IR24) were put into preliminary yield trial (12 x 4 RGB, plot size 9' x 30') at Hmawbi.</td>
</tr>
</tbody>
</table>
- on the basis of field observation, 5 lines (3 from 25 kR, 2 from 30 kR) were selected for higher plant height and superior grain quality. Harvesting of grains was effected due to heavy and continuous rain and yield data were not analyzed.

- 55 single plants were selected on the basis of higher plant height and superior grain quality from the 6 lines (35 kR bulk) and 19 single plant progeny lines (35 kR).

1973 (wet season) M5

- 5 strains (3 from 25 kR, 2 from 30 kR) were put on yield trials at Gyogon, Hmawbi, Mandalay and Pegu, along with 3 checks (IR5, IR24, and C 4-63). The experiments at Mandalay and Pegu were damaged and no data were collected. The design was a 8 x 4 RCB with plot size 7' x 30'.

Average results are given below

<table>
<thead>
<tr>
<th>Var.</th>
<th>days to maturity</th>
<th>pl. ht. (cm)</th>
<th>1000 grain wt. (gm)</th>
<th>grain type</th>
<th>yield/ acre(lb)</th>
<th>Milling out-turn (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 4-63</td>
<td>122</td>
<td>108.3</td>
<td>20.2</td>
<td>A</td>
<td>3550</td>
<td>58</td>
</tr>
<tr>
<td>IR24</td>
<td>112</td>
<td>94.5</td>
<td>21.1</td>
<td>A</td>
<td>3284</td>
<td>58</td>
</tr>
<tr>
<td>IR5</td>
<td>144</td>
<td>119.2</td>
<td>23.1</td>
<td>C</td>
<td>4182</td>
<td>52</td>
</tr>
<tr>
<td>Mutant-1</td>
<td>147</td>
<td>135.6</td>
<td>22.8</td>
<td>A</td>
<td>4508</td>
<td>65</td>
</tr>
<tr>
<td>Mutant-2</td>
<td>135</td>
<td>126.3</td>
<td>22.3</td>
<td>A</td>
<td>4155</td>
<td>58</td>
</tr>
<tr>
<td>Mutant-3</td>
<td>144</td>
<td>133.5</td>
<td>21.9</td>
<td>A</td>
<td>4202</td>
<td>58</td>
</tr>
<tr>
<td>Mutant-4</td>
<td>136</td>
<td>125.1</td>
<td>21.8</td>
<td>A</td>
<td>4134</td>
<td>58</td>
</tr>
<tr>
<td>Mutant-5</td>
<td>138</td>
<td>128.7</td>
<td>22.6</td>
<td>A</td>
<td>4528</td>
<td>65</td>
</tr>
</tbody>
</table>

LSD (yield) = 312.2 lbs (p=0.05)

The above results indicated that Mutant-5 (25 kR) and Mutant-1 (30 kR) were significantly better in yield than all the checks and superior in grain quality than IR5. The other mutants (Mutant-2 - 30 kR, Mutant-3+4-25 kR) were equal in yield with IR5 but significantly better than C 4-63 and IR24. The grain quality was superior to IR5.

Taking these factors into consideration, Mutant-5 was recommended for multiplication and was released in 1975 for mass production. The mutant strain was named as SHWE WAR TUN.

3. Earliness (flowering date and ripening time)

Earliness is a very important plant breeding objective and much success has been achieved in inducing earliness through mutagen treatments in otherwise high yielding varieties of rice.

The fact that early heading mutants can be detected with ease from a big population and that the variants for heading time are more frequent after
mutagen treatment than chlorophyll mutants (Yamagata 1964), put the mutation breeding in an advantageous position over other methods. Yamagata further reported that to a certain degree the mutation spectrum depends on the genetic constitution of the variety used. He observed that by treatment of early maturing varieties comparatively more late maturing variants were obtained and vice versa.

Variations of flowering date and maturity time are in parallel relations in general but departures from such relationship are also observed. The difference for days to flower between the mother variety and the early mutant may not be the same as for days to maturity. In the latter case a smaller difference is often observed. While selecting early mutants, particularly in case of wide differences in maturity periods, the per day production may also be considered. Under certain circumstances, early mutants differing by 2 - 3 weeks in maturity having higher per day production and a total yielding ability only slightly lower than the mother variety, may be of economic significance, as early strains would release the land earlier and can fit into intensive cropping systems.

Mikaelsen et al. (1971) developed an early maturing, high yielding variety, Early Cesariot, later named "Nucleoryza", from a high yielding, late maturing, disease resistant French variety which did not do well in Hungary due to its very late maturity. The mutant was not only extremely early maturing and disease resistant, but it also gave the highest yield as compared to the best rice varieties and new breeding lines in Hungary. In our work with the variety IR8 we also have been able to establish mutant strains having 2-4 weeks earlier maturity than the mother IR8 but equal or even higher yield.

The methods followed for developing the above mutant varieties are given below:

1. Mikaelsen et al. (1971)
   - Variety: French variety Cesariot (late maturing, disease resistant)
   - Mutagen: Gamma rays 10, 15, 20 krad.
   - Fast neutrons 1000, 1500, 2000 rads.
   - M1 (1966): 1000 seeds irradiated with each dose and grown.
   - Almost 1000 panicle progenies were grown from each dose.
   - A number of early flowering plants were observed and harvested.
   - One of these mutants from fast - neutron irradiation was extremely early and headed 3 weeks earlier than mother variety and was selected.
   - M4 (1969): All the seeds from the above mutant line were propagated and it continued to breed true. The mutant was called Early Cesariot.
   - M5 (1970): The Early Cesariot was included in varietal trials in two locations along with best rice varieties and new breeding lines in Hungary.

   The promising results obtained in these trials indicated that this mutant could be used as a new variety. The mutant was officially approved in 1972 and recommended for cultivation under the name "Nucleoryza".

2. Haq et al. (1971)
   - Variety: IR8, high yielding introduced variety. Under Bangladesh conditions it was comparatively late maturing in both AUS and BORO seasons.
   - Mutagen: Gamma rays, 30 kR.
   - M1: Approximately 100,000 plants were grown.
   - Selected at random 300 normal looking fertile plants.
Mp - 300 plant progenies were raised - 20 plants per progeny.
- A bulk population of approximately 100,000 plants was also grown.
- Selected 4 early maturing mutants from the bulk.

M₃ - The 4 early maturing mutants were grown in larger plots - 20 ft x 5 ft. The mutants were found to be breeding true for earliness.

M₄, M₅, M₆ - Yield trials were conducted along with IR8. The mutants were maintaining the early maturing trait and comparable yield, even under different growing season, in respect to mother IR8. Mutants 38 and 39 were earlier by 21-25 days and mutants 20 and 24 were earlier by 10 days. From the performance of the yield trials mutant Nos. 24 and 38 were found to be fairly satisfactory and they were named and released in 1971-72 as IRATOM 24 and IRATOM 38 varieties. IRATOM 38 had higher protein content (2% more) than IR8.

In an evaluation trial at IRRI in 1971, IRATOM 24 recorded the highest yield (6.40 t/ha) amongst 52 entries which included mutant strains from different countries and IRRI varieties and selections. IR8 yielded 4.90 ha.

4. Disease resistance

Breeding for disease resistance is a very important plant breeding objective and mutation breeding may have an advantage over other methods, particularly when such improvement is related to a well adapted high yielding variety. With the availability of efficient screening techniques the problem of detection of mutants showing resistance became easier. Although, the frequency of disease resistant mutants is low, it should not be a limiting factor with rice crop, as a large population can be handled without much difficulties for screening purpose.

Amongst the rice diseases, blast (Piricularia oryzae) and bacterial leaf blight (Xanthomonas oryzae) are the most serious and induced mutation breeding has proved to be successful in producing resistance in high yielding varieties as may be observed in the following examples.

Variety - Maratelli; An early maturing and high yielding variety but highly susceptible to rice blast fungus.
Mutagen - Gamma rays, 25 kR.
M₁ and subsequent generations were grown at Vercelli, Italy.
M₂ - M₄ - Grown as bulk under natural selection pressure and then about 2000 lines were selected.
M₆ - 178 lines were grown which had improved blast resistance. Their foliage was free from blast while the mother variety was completely burnt and killed due to disease infection. Some mutant lines had the same maturity time as Maratelli or were earlier, but most of resistant lines were maturing later.

The ultimately selected lines were twice tested at IRRI by Dr. S.H. Ou under heavy infective conditions. Three of the lines were highly resistant in both the tests. One of these mutant strains were multiplied for extension in Italy as an improved cultivar, "Fulgente".

2. S. Kaur, S. Padmanabhan, P. Kaur (1977)
Variety - Ratna (IR8 x TKM6) a high yielding rice variety but susceptible to blast disease.
Mutagen - EMS, 150 dehusked seeds for each dose were pre-soaked in distilled water for 4 h. and then treated with aqueous solution of 0.1% and 0.2% EMS for 6 h. After treatment the seeds were thoroughly washed in running water.

M<sub>1</sub> - The treated seeds were germinated first in petridishes and then transplanted in trays with close spacing to reduce tillering. At maturity several panicles per plant were harvested. 80 plants survived, 44 from 0.1% and 36 from 0.2% treatment.

M<sub>2</sub> - 100 seeds from each plant were sown in nursery beds. Total M<sub>2</sub> population 100 x 80 = 8000. A row of Co 13, a susceptible variety, was planted between the test rows. Ammonium sulphate (40 kg/ha) applied before planting.

M<sub>3</sub> - 300 resistant plants selected.

M<sub>4</sub> - The selected plants were progeny-tested in a uniform blast nursery. 100 resistant plants were selected.

M<sub>5</sub> - 1000 plant progenies tested and 1500 plants selected. M<sub>5</sub> - 1500 selections progeny tested and 50 selections bred true.

Remarks: The data indicated that the resistant and the susceptible selections of M<sub>2</sub> and M<sub>4</sub> segregated in the M<sub>5</sub> generation but the percentage of resistant plants in selections for resistance, gradually increased from 23.6 in M<sub>2</sub> to 31.0 in M<sub>4</sub> and 44.3% in M<sub>5</sub>. Mutants similar to Ratna in grain characters with high yield and improved resistance have been obtained.

In an attempt to induce resistance to bacterial leaf blight in the high yielding variety - Vijaya, they could not recover any true breeding resistant line with high yielding ability like Vijaya.

5. Quality (protein content)

Breeding for increased protein content is an extremely important objective. Rice is the staple food of most of the worlds under privileged people and thereby a major source of food protein where the daily intake of rice is high. Its value as a protein source is enhanced by its high lysine content relative to other cereal grains. The main limitation of rice, as a protein source, is its low protein content and this is particularly true with high yielding varieties. An increase in protein content will increase intake of protein, provided the quality of the protein is not impaired, in this way it may be feasible to combat malnutrition which is a burning question with the lower economic levels of the population.

Conscious screening for better nutritional value has only recently been started and research in this line has received accelerated impetus from the seed protein improvement programmes under joint sponsorship of FAO/IAEA Division of Atomic Energy and Gesellschaft für Strahlen- und Umweltforschung (GSP), (FRG).

Protein estimation in a world collection of rice consisting of 7760 entries at the International Rice Research Institute (IRRI) indicated a range of 5.3 to 9.3% in japonica varieties and 7.6 to 13.8% in indica varieties (Juliano et al. 1968). Tanaka (1969 a,b, 1971) has reported recovery of protein content 2.5 times higher than the mother line in early high yielding mutants after gamma irradiation. In 545 mutant lines from Norin 8 (protein content of brown rice = 6.54%) a range from 4.2. to 16.3% protein was found. In one mutant the stature was shorter; it flowered earlier and had high yielding capacity with protein content of 13-15 percent.

Narahari and Bhatia (1975) reported that protein percentage in dehusked kernels of IR8 mutants (diethyl sulphate) ranged between 7.3 and 10% as against 7.9 - 9.4% in the control.
These two examples show that induced mutation breeding can play a role in seed protein improvement in rice. The range of variation after mutagen treatment of one variety exceeded the range of variation observed in the world collection.

Wheat breeders have indicated that there exists a high positive correlation between absolute amount of protein in the seed and seed-size (Jain 1976) and between 1000 grain weight (+ 0.86) and protein yield per hectare (Rtbellen 1977). In our study we observed that the earlier maturing mutant varieties IRATOM 38 and IRATOM 39 developed from IR8 had ca. 2% higher protein content. Narahari and Bhatia (1975) have observed a negative correlation between seed protein content and grain yield, grain weight and plant height.

Tanaka (1976) has been successful in isolating and developing high yielding rice variants with high protein productivity per unit area and the method he used and the recommendations he made are outlined below:

<table>
<thead>
<tr>
<th>Variety</th>
<th>Nihonbare (low land variety, protein content 8.0%, widely cultivated).</th>
</tr>
</thead>
<tbody>
<tr>
<td>M&lt;sub&gt;1&lt;/sub&gt;</td>
<td>1000 air dried seeds treated with gamma rays of 20 and 30 krad.</td>
</tr>
<tr>
<td>M&lt;sub&gt;2&lt;/sub&gt;</td>
<td>220 panicle progenies from 220 M&lt;sub&gt;1&lt;/sub&gt; plants with high seed fertility in each treatment were raised by pedigree method.</td>
</tr>
<tr>
<td>M&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Seeds from 3300 M&lt;sub&gt;2&lt;/sub&gt; plants for each treatment were analysed for protein content by the Kjeldahl method.</td>
</tr>
<tr>
<td>M&lt;sub&gt;4&lt;/sub&gt;</td>
<td>201 high protein variants (more than 11% protein in brown rice) were isolated regardless of any visible changes in mature plants. Most of them were malformed ones.</td>
</tr>
<tr>
<td>M&lt;sub&gt;5&lt;/sub&gt;</td>
<td>From 201 variants 37 lines that did not segregate for visible mutations and had similar phenotypes to the mother were selected.</td>
</tr>
<tr>
<td>M&lt;sub&gt;6&lt;/sub&gt;</td>
<td>37 lines were raised - 15 plants per line.</td>
</tr>
<tr>
<td>M&lt;sub&gt;7&lt;/sub&gt;</td>
<td>After protein analyses 11 lines were selected which had significant increase in protein content (9.2% against Nihonbare 7.9%).</td>
</tr>
<tr>
<td>M&lt;sub&gt;8&lt;/sub&gt;</td>
<td>The 11 lines were raised as individual families, each family consisting of 15 lines with 15 plants and with one replication.</td>
</tr>
<tr>
<td>M&lt;sub&gt;9&lt;/sub&gt;</td>
<td>After discarding 4 families (low yielding and segregating) 7 families consisting of 12 lines were isolated as high protein mutant lines (more than 9.8% against 8.4 of Nihonbare). After the M&lt;sub&gt;4&lt;/sub&gt; generation, protein productivity of these 12 lines was successively examined at several stations and under various environmental conditions. On the basis of these trials a few lines having increased productivity was isolated.</td>
</tr>
</tbody>
</table>

He concluded: "(a) Screening of high protein variants in earlier generations must be made on normal appearing plants, because most of the high protein variants resulted from decreased starch synthesis ability due to malformed plant types and/or sterility; (b) although some of the isolated lines, as high protein mutants, had minor changes in visible characters, they maintained superior agronomic traits of the mother variety; i.e. an increase of protein content was not always accompanied with inferior changes of other characters; (c) high protein lines having 10 - 20% increase in protein productivity per a unit area could be isolated from Japanese varieties through mutation breeding; however, mutant lines with extremely increased protein content and grain yield can hardly be obtained by a single mutagen treatment, because both the characters are not always controlled by major genes".

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CONCLUSION

The extensive research work that has been conducted and the useful results that have been achieved with rice, within last two decades, leave no doubt that induced mutation breeding is a very useful method for the improvement of rice crop covering various aspects. By now in many research institutions this method has been accepted as an integral part of the breeding programme.

For successful exploitation of this method the breeder must have clear cut aims and for assured success the objectives should be limited to easily detectable characteristics or for which quick and efficient screening techniques are available. It has been established that increase in variability through induced mutation is easily achieved, but the detection and isolation of mutants are a rather difficult task. Characters such as maturity time, disease resistance, stiff-straw, erect habit, plant height are visually detectable. Quick screening for protein content is also available. For these aims mutation breeding appears to have an advantage, as may be observed from the examples cited where mutants have been released as improved varieties. In case of selecting for yielding ability, selection on the basis of plant type (erect habit, short-culm, stiff straw, lodging resistance) which may be connected with yield has given satisfactory results. This is particularly true with the tall indica varieties. Success has also been achieved in modifying certain characteristics of the high yielding rice varieties to make them adaptive and acceptable to the growers and consumers. Sterility is a problem in irradiated population and good results have been achieved by selecting in M₁ primarily the normal looking and highly fertile panicles.

Lastly, it may be stressed that, in general, a reasonably large plant population both in M₁ and M₂ are very important for success in mutation breeding work; although in a few cases fairly satisfactory results have been achieved with small M₁ population.

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ABSTRACT

The present position of sugar industry particularly cane sugar production in the world has been discussed. The role of African Countries which can contribute more than the present 11% to world cane sugar production is presented. The breeding methods employed in cane growing countries indicate the biparental crossing and selection in $F_1$ has been the major method used to develop varieties. Due to cytogenetical peculiarities, thousands of seedlings are grown to select the desirable genotype.

Mutations or sports has been a source of variation for selection in nature. Induced mutations have only enhanced the mutation rate and has enabled the plant breeders to get better variation for selection. Though many mutagens have been used gamma rays have been most effective. Induced mutations for non-flowering, spineless leaf-sheath, higher sugar content, yield and resistance to diseases like smut and downy mildew have been reported. The methods of making mutated tissues express itself have been indicated. Mutation breeding holds out promise in sugarcane in that the basic variety or genotype can be kept intact and a few characters changed as desired by the plant breeder provided proper selection methods are employed.

1. INTRODUCTION

The major sources of sugar are sugarcane and sugar-beet. The minor sources are Palms, Sorghums, Maple and Pineapple. During 1977-78, the world sugar production has exceeded 90 million tons (92.62 m/tons) and consumption is around 87 million tons. However this picture of overproduction is bound to

* Part of research reported in this paper has been carried out under IAEA Research Contract No. 1680.
change for consumption with increasing population. Sixty per cent of this has come from sugarcane. In years to come also, only cane areas can be expanded and cane sugar would continue to be the major source of sugar.

2. **PRODUCTION IN AFRICA**

Sugarcane is essentially a tropical crop and hence the developing countries of Africa, South America and Asia will have to play a major role in increasing cane sugar production. About 94 countries in the world cultivate sugarcane and among these, 28 African countries take part (Table 1). In spite of this large number there are only 5 African countries namely Belize, South Africa, Swaziland, Malawi and Uganda export sugar as per International Sugar Agreement signed in Geneva in January, 1978. Countries like Kenya, Nigeria are importing members of this agreement. A survey shows that most of the African countries have taken to sugarcane cultivation only after 1970. According to International Sugar Journal (1978), African continent contributed only about 11.2% to World sugar production. Hence there is considerable scope for increasing sugar production in this part of the world. This increase can be mainly brought about by area expansion and breeding new varieties that can yield high sugar per unit area per unit time and adopting improved agromonic and cultural practices.

3. **BREEDING NEW VARIETIES**

In recent years, it has been shown that there is considerable genotype x environment interaction in varieties, thereby indicating that a variety or varieties yielding well in one location do not yield the same in another location where soil are different. A survey of varietal position in the past and present supports this contention in that there have been very few varieties like "W Co 310, POJ 2878, Co 419 and B 37132 which can be called "Universal". In this context, breeding varieties for specific locations becomes the most important problem. Hence, in all the countries, especially in Africa where sugar industry
is being expanded not only to increase production but also to open up employment potential for thousands of farmers and others employed in many ancillary industries like distillation, paper board manufacture, etc., breeding and development of varieties is the pivot of this second largest industry in the world next to textiles. The industry will collapse if the cultivators do not provide enough raw materials (canes) with satisfactory sugar recovery in order to run the mills with profit and the farmers can get a good return for their produce.

A survey of sugarcane breeding in the world shows that varieties have been mainly developed for increasing yield, sugar content and resistance to local diseases and pests. Sugarcane has the dual advantage of producing true seeds called "fuzz" or "fluff" which can be used for genetic studies, breeding purposes and propagation by cuttings once the variety is produced. However, for geneticists and plant breeders, the crop presents problems because of its high polyploid nature, heterozygosity, autosyndetic pairing of chromosomes and other cytogenetical peculiarities (3). Hence breeding has been mostly confined to selection in F₁ generation after crossing two parents having desirable characters. Though it is said that mutation is a single cell event and "The possibilities of mutation breeding in vegetatively propagated plants stand or fall by the availability of such a method" (8), it can be safely mentioned that this need not always be the case. For example in sugarcane and in many of the vegetatively propagated crops, all somatic cells do not have the same chromosome number. If the cell from which the plant develops has a different chromosome number (different from the number which occurs in highest frequency), then the genotype of the plant would not be the same as parent variety. This would complicate the results.

In a multicellular system, before the mutated cell forms a constant periclinal layer through growth and propagation, it will show a sectorial and mericlinal distribution in the shoot developing from the treated bud. The types of chimeras
produced by irradiation or Colchicine treatment have been studied in tomato plants (9) heterozygous for recessive chlorophyll deficiency. Usually the mutated tissue was localised to the basal leaves of young plants and with increasing leaf number from the base, the mutated area becomes much larger but rarer.

It has been the experience of several breeders that mutated tissues will mainly be limited to the basal buds of the the variability and selecting from these induced mutations and chimeras. In nature, only some of the mutations would have survived whereas in experiments, the desirable sports could be selected.

It has been commented (1) that all mutations are single cell events so that the outcome of any somatic irradiation is always a chimera which will be mericlinal at first and if it survives in a stable form, finally periclinal. It is very difficult to agree to this point that all mutations are single cell events. In sugarcane, the anatomical studies of dormant and germinating buds made earlier (2) and during the last few years at this Institute by the author and his colleagues have clearly shown that stalks or canes arise from buds which have initials (15 to 20 cells) already present in the dormant bud at the time of irradiation. Hence the target being many cells, only one cell cannot be the source of mutations. On the contrary, it has been clearly shown (25) that it is seldom possible to get one complete mutated clone in \( W_1 \) or \( W_2 \) generation and in all the cases, only one or two canes or at times only part of the stalk is mutated or changed. Further decapitation experiments have shown that after decapitation on 60th or 75th day, there is considerable increase in frequency and spectrum of changes (29) indicating that the mutated cells are able to express themselves after decapitation (diplontic selection is overcome) which would not have been the case if mutation had been a single cell event and would have been lost in intrasomatic selection.

The limited success of induced mutations in sugarcane in the past has been mainly due to inadequate screening methodo-
logy, propagation and selection in a systematic way to get stable mutants which would not revert back. Improving the existing commercial varieties for one or two desirable characters has hardly been successful because of lack of follow up of mutation generations up to $nM_4$ or $nM_5$ till stability is established. Very little information is available on the efficiency and effectiveness of physical mutagens such as gamma rays, X-rays, neutrons. Chemicals have been rarely used because of penetration problems and large quantities required for experimental work. Lastly the production of chimeras in mutagen treated plants further complicated the selection and this ended up in many of the scientists not getting desirable mutants. It is well known that only some types of periclinal and sectorial chimeras are stable if proper selection methods are employed. The ideal condition for mutation breeding is where the entire plant arises from a single cell. Such a situation was obtained in Saintpaulia (6) Streptocarpus (7). In countries like India, Australia, Java, Barbados, other breeding methods like inbreeding, backcrossing (nobilisation) have also been used (21). In early 1900's, the crossing of S. officinarum with S. spontaneum (wild relative) and repeated backcrossing to noble canes which produced the famous FOJ series and Co varieties which revolutionised the sugar yields and saved the industry from virtual collapse in countries like Java and India. In view of the success in these breeding techniques and limited knowledge of genetic architecture, selection in $F_1$ has continued to be the major method of breeding new varieties. It takes about 8 to 10 years to release a variety to the cultivator from the time a cross is made provided a desirable recombinant has been selected. Hence plant breeders were searching for newer methods which would reduce this time, create new variability without altering the basic genotype.

4. **INDUCED MUTATIONS**

The sugarcane flower was thought to be infertile and the only source of variation was believed to be sports (mutations or chimeras). However, after 1950's, induced mutations
became a tool to plant breeders when many radiation sources and radiomimetic substances became available. Plant breeders and geneticists immediately jumped into the possibility of increasing primary shoot. Many mutations are lost as these buds in plants with apical dominance often refuse to break or grow further. Also the appearance of mutations in the form of sectorial chimeras makes them difficult to detect especially the less drastic ones and hence most interesting ones. It has been observed that cutting the primary shoot back close to the irradiated scion and forcing the adventitious buds to develop resulted in high rates of mutations for various characters like early harvesting types, increased fruit weight, sugar content and frost resistance in sour cherries. In sugarcane, when the irradiated growing buds were decapitated to get over apical dominance, there was an enormous increase in the frequency and types of changes observed in canes (Table II) developing, thereby confirming the theory that many mutations are lost due to apical dominance.

5. NATURAL MUTATIONS

Mutations for easily distinguishable characters such as stalk colour have been common in sugarcane for very long time (14). It is not known to what extent such vegetative mutations take place in the invisible but economically important characters such as yield factors and sugar content of juice. It is also known that increase in sugar content in some of the species could have been due to natural mutations and some of these were selected by natives for sweetness and chewing (15).

Attempts to first induce mutations was reported by Experiment Station of Hawaiian Sugar Planters Association as early as 1928. Sugarcane varieties H 109 and striped tip were treated with X-rays. In 1933, sugarcane breeders of Taiwan Sugar Experiment Station picked up a bud sport from a commercial variety F 108. The mutant F 1108 outyielded its parent variety. It has 114 chromosomes and parent 111 (17). Both did not differ in resistance to mosaic disease.
Two possible approaches for the application of mutation breeding in sugarcane have been suggested (16). Firstly by subjecting the existing varieties to mutagenic treatments and then practising selection in subsequent vegetative generations. Secondly incorporating induced mutations in the gametes which combine when desirable parental clones are crossed in the first and second sexual generations as a part of breeding programmes. Segregation, recombination and selection in successive sexual generations would result in perpetuating desirable combinations and eliminating undesirable ones.

The approach suggested above that existing varieties should be treated with mutagens is quite valid and all the plant breeders in sugarcane who have used induced mutations as a tool have adopted this method. However, the second suggestion that induced mutations could be incorporated in the gametes and subsequently selected may not hold the ground because of high polyploidy and heterozygosity of this crop and its cytogenetical peculiarities. Further it is difficult to know whether the observed changes in the genotype are due to mutation or recombination, since even in normal crossing and raising of F₁ seedlings, no two seedlings resemble one another even if lakhs of seedlings are raised unlike other cereal crops.

There have been several reports of induced changes in stalk characters (14, 15, ), foliar abnormalities (17). However, many of these mutants including the red rot disease resistant ones (25) either did not repeat in subsequent generations or broke down due to basic chimeral nature and inadequate selection methods adopted to evaluate their value as basic genetic material that can be used in breeding programmes (26) or as a variety with added stable desirable character more valuable to the cultivator than the parent variety.

6. MUTANTS OF ECONOMIC VALUE

There have been quite a few economically useful mutants reported from several countries. A few of them have reached com-
mercial cultivation. However, in many after the first reports little information has been published about their stability, performance in large scale trials and extent of area occupied by the new mutant. It could be presumed from the personal communication of the author that many of them are in final testing trials since it takes quite a few years to multiply them.

Non-flowering mutants have been reported in several varieties (18, 19, 20). Flowering is an exceedingly complicated terminal growth (1) and should be susceptible to blockage at several or even many points along the line. However, it should be pointed out that it may be easy to induce a mutant for a non-flowering character but the mutant should also have in tact all the other characters like sucrose content, yield and resistance to diseases and pests. The mutants reported from Barbados have been economically valuable in that all other important characters are in tact. Both B 52107 and B 49119 have given non-flowering mutants (22). However, in N Co 310, no non-flowering mutants could be isolated in gamma ray irradiated material in Queensland though non-flowering mutants were reported in 3 sub-clones of N Co 310 earlier (1). High sugared and non-flowering mutants were reported in 8 sub-clones of P 146, P 160 and N Co 310 (14). Non-flowering have also been obtained in Co 527 (24). These mutants are of interest since in tropical belt within 15 degrees of equator, heavy flowering of sugarcane is frequently the reason for rejecting an otherwise satisfactory variety. Flowering is undesirable since initiation occurs at least 3 months before harvest, in the middle period of maximum growth. Flowering stalks show progressive deterioration and in extreme cases, become useless for processing. Yield potential is therefore severely limited, even though heavy flowering is often associated with vigorous early growth.

Mutants for glabrous leaf sheath in pubescent variety Co 527 (23, 30) are of value where manual harvesting is in vogue. These mutants occupy large areas where the parent varieties were cultivated and are replacing them in India and Pakistan. Fortu-
nately the mutants have the same sucrose content, yielding ability, disease and pest resistance as the parent variety.

As many as 52 stable mutants in several commercial varieties have been obtained based on selection method outlined earlier and are maintained at Sugarcane Breeding Institute, Coimbatore, India. These mutants have several induced changes with basic genotype intact. To mention a few examples, a vigorous fast growing mutant has been isolated and stabilised after 4 to 5 years of selection in Co 527, an early maturing commercial variety (27). The mutant grows faster, has more number of millable canes per unit area and same juice qualities of the parent variety. In addition, it also yields 40% more (Table III) than the parent variety. A cytological study has shown absence of 2 to 3 somatic chromosomes indicating that this is a chromosomal mutant. Fortunately the chromosomes having growth and cane formation inhibiting factors/genes have been deleted to the advantage of the plant breeder. This mutant has been released for cultivation. This mutant is an example as to how chromosomes having undesirable genes can be eliminated by mutagenic treatments and subsequent selection of mutagen treated populations for 4 to 5 years can yield desired objectives.

A mutant in the variety Co 419 which has higher germination, early shoot population and higher number of millable canes/unit area has also been isolated and stabilised (29). This mutant has gone into yield trials in 1978.

7. DISEASE RESISTANT MUTANTS

Mutations for resistance to diseases and pests are the most desired changes by plant breeders in this crop since high polyploidy and heterozygosity have been stumbling blocks for backcross breeding. It has been possible to obtain mutants for mosaic resistance (Kebonagung strain) in Java in variety POJ 3016 by the use of gamma rays (33). Smut is one of the most widespread diseases in cane growing countries of the world. In African Countries, smut, mosaic and leaf stripe are some of the important diseases. Though it is possible to control these diseases, there
is no substitute to cultivation of resistant varieties. A survey shows that smut resistant mutants have been obtained in varieties Co 1287 and Co 740 in India (29). Co 740 is the "wonder" cane of India and yields as high as 100 tonnes per acre with a high recovery of 12% and very good ratooning ability. However, this variety is highly susceptible to smut and may go out of cultivation in years to come. A back-crossing and mutation breeding programmes are in progress to incorporate smut resistance to this variety. Though back-cross breeding has given resistant types, these do not resemble the parent variety Co 740 in toto and are not in par with it in yield and ratooning ability. As many as 200 smut resistant mutants have been obtained in gamma ray treatments. They have been tested for 3 years for stability of the character. They also resemble the parent variety in most of the characters. These mutants are in trials along with the parent variety. There is a distinct possibility of obtaining smut resistance mutants with parent genotype Co 740 in tact.

Smut resistant mutants have also been reported in Co 528 from Pakistan (35) and in varieties F 146, F 173 and F 177 from Taiwan (17, 18). The Taiwan varieties are wellknown for their superiority. However, the cultivation of them has been restricted to some areas only because of their high susceptibility to smut. These mutants would further extend their areas of cultivation. Mutants resistant to downy mildew (Sclerospora sacchari) have been obtained in the variety F 160. The mutant showed 1.92% susceptibility whereas the parent 9.62%. Mutants for high sugar content have also been obtained in F 160. A mutant with reduced leaf sheath hairiness was also isolated in variety F 172 by gamma rays.

8. SELECTION OF MUTANTS

The dosage of gamma rays used in all the experiments have been between 3 and 5 krad. Rarely higher doses have been used. Single eyed or budded setts have been used. The follow up of irradiated material from first vegetative mutation generation onwards on individual stalk basis is essential for isolation
and selection of desired mutants. Most of the induced mutations are either mericlinal, periclinal or sectorial chimeras and hence continued selection for 4 or 5 vegetative generations for the character desired till stability is obtained is essential.

The achievements of sugarcane mutation breeding during the last 20 years has been brought out in this review. Though success has been limited, the prospect for new advancement in varietal improvement through the use of induced mutations appears bright. As indicated in this review, the possibility of inducing mutants for higher germination, growth rate, earliness, higher yield and sucrose content, resistance to diseases like smut, mosaic, downy mildew and host of other characters appears bright provided proper selection of mutagen treated population is made.

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**Table I**

**AFRICAN COUNTRIES PRODUCING SUGAR CANE**

<table>
<thead>
<tr>
<th></th>
<th>Country</th>
<th>Production</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Angola</td>
<td>60,000</td>
</tr>
<tr>
<td>2</td>
<td>Cameroon</td>
<td>54,000</td>
</tr>
<tr>
<td>3</td>
<td>Chad</td>
<td>15,000</td>
</tr>
<tr>
<td>4</td>
<td>Congo (Brazzaville)</td>
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</tr>
<tr>
<td>5</td>
<td>Egypt</td>
<td>6,90,000</td>
</tr>
<tr>
<td>6</td>
<td>Ethiopia</td>
<td>1,52,000</td>
</tr>
<tr>
<td>7</td>
<td>Ghana</td>
<td>13,000</td>
</tr>
<tr>
<td>8</td>
<td>Ivory Coast</td>
<td>54,000</td>
</tr>
<tr>
<td>9</td>
<td>Kenya</td>
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</tr>
<tr>
<td>10</td>
<td>Madeira</td>
<td>2,000</td>
</tr>
<tr>
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<td>Malagasy Republic</td>
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</tr>
<tr>
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<tr>
<td>13</td>
<td>Mali</td>
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</tr>
<tr>
<td>14</td>
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</tr>
<tr>
<td>15</td>
<td>Morocco</td>
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</tr>
<tr>
<td>16</td>
<td>Mozambique</td>
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</tr>
<tr>
<td>17</td>
<td>Nigeria</td>
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</tr>
<tr>
<td>18</td>
<td>Reunion</td>
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</tr>
<tr>
<td>19</td>
<td>Rhodesia</td>
<td>2,90,000</td>
</tr>
</tbody>
</table>

---
20. Senegal 40,000  
21. Somalia 38,000  
22. South Africa 23,40,000  
23. Sudan 2,29,000  
24. Swaziland 2,39,000  
25. Tanzania 1,20,000  
26. Uganda 25,000  
27. Upper Volta 30,000  
28. Zaire 70,000  
29. Zambia 85,000  
Total 62,76,000  

Table II  
EFFECT OF DECAPITATION ON THE FREQUENCY OF MORPHOLOGICAL CHANGES IN VM

<table>
<thead>
<tr>
<th>Variety</th>
<th>Dose in Krad</th>
<th>Percent 60 days</th>
<th>Percent 75 days</th>
<th>Stools when decapitated at 90 days</th>
<th>Stools when decapitated at 105 days</th>
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<td>Control</td>
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<td>0</td>
<td>15.0</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>33.3</td>
<td>21.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0</td>
<td>17.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co 419</td>
<td>Control</td>
<td>10.5</td>
<td>15.9</td>
<td>10.0</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>21.1</td>
<td>40.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>46.7</td>
<td>50.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co 453</td>
<td>Control</td>
<td>5.0</td>
<td>0</td>
<td>5.0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>31.6</td>
<td>29.4</td>
<td>11.1</td>
<td>17.6</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>22.2</td>
<td>35.3</td>
<td>21.1</td>
<td>17.6</td>
</tr>
<tr>
<td>Variety</td>
<td>8 months</td>
<td>9 months</td>
<td>10 months</td>
<td>11 months</td>
<td>No. of</td>
</tr>
<tr>
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<td>-----------</td>
<td>-----------</td>
<td>--------</td>
</tr>
<tr>
<td></td>
<td>Brix</td>
<td>Puri-</td>
<td>Brix</td>
<td>Puri-</td>
<td>Brix</td>
</tr>
<tr>
<td></td>
<td>Suc-rose ty</td>
<td>Brix</td>
<td>Suc-rose ty</td>
<td>Brix</td>
<td>Suc-rose ty</td>
</tr>
<tr>
<td>Co 527</td>
<td>14.67</td>
<td>12.18</td>
<td>82.86</td>
<td>17.56</td>
<td>15.19</td>
</tr>
<tr>
<td>M-10</td>
<td>14.64</td>
<td>11.97</td>
<td>81.67</td>
<td>18.81</td>
<td>16.59</td>
</tr>
<tr>
<td>F.I-2</td>
<td>18.51</td>
<td>16.41</td>
<td>88.62</td>
<td>19.64</td>
<td>17.70</td>
</tr>
</tbody>
</table>
Mutation Breeding in Groundnut at Trombay

S.H. Patil and Chandra Mouli
Biology and Agriculture Division
Bhabha Atomic Research Centre
Bombay 400 085, India

Abstract

India is producing about 1/3 of all groundnuts in the world, but only negligible amounts are exported. Large quantities of deoiled cake for animal feed, however, exported to Europe and Japan bring India 50-60 million US$ in foreign currency. Groundnut production is based upon a limited number of varieties, some of which are more than 50 years old. New improved varieties are desired. Mutation breeding of groundnut taken up at BARC Bombay has resulted in a number of varieties with high yield potential, large kernels and/or improved oil content.

Introduction:

India is the largest producer of groundnut in the world contributing over 33% to the global production. The other important producers are China, USA, Senegal and Nigeria. Before 1960, nearly 5% of the produce from India was sold in the international market. In the recent years the export of groundnut has dwindled to a negligible quantity, though still there is great demand of Indian groundnuts for 'table purposes'.

For the country groundnut is an important oilseed crop. It occupies an area of over 7 million hectares with a production of about 6.0 million tons. The average yield is around 850 Kg/ha as the crop is mainly rainfed. Production is low, if the rainfall is less or not properly distributed during the cropping season. Fluctuation in groundnut production influence the availability and price of edible oil in the market.

The role of groundnut as a food crop is increasingly recognised especially due to higher content of digestible proteins in the kernels. Besides being milled for oil

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extractions large quantities of kernels are consumed as roasted nuts as well as used in the culinary preparations. Deoiled cake used in animal feeds is exported to Europe and Japan from India to earn 50-60 million US dollars annually. Thus groundnut crop plays a vital role in the economy of India.

Groundnut is grown in India since two centuries. Yet a limited number of varieties are in cultivation indicating lack of adequate variability. Some of the present cultivars are in cultivation for more than 50 years. New varieties with high yielding ability are, therefore, required to improve groundnut production.

**Mutation Breeding:**

Mutation breeding in groundnut was initiated two decades ago at the Bhabha Atomic Research Centre (BARC), Bombay, with a view to generate variability in characters contributing to economic yield. A popular cultivar, Spanish Improved, was used in the experiment. After studying the morphological effects of 10-75 kR X- and gamma irradiation (1 & 2), cytological mutants were isolated (3). More mutations were obtained in 35-45 kR dose range. Intensive studies in the progenies of meiotically aberrant $M_1$ plants showed not only increased occurrence of mutations, but also their appearance in later generations (Table I). It might be emphasized that more than 40% of the mutants were induced in the two cytological $M_1$ variants suggesting that increased number of mutations could be obtained in the progenies of cytological mutants (4).

In the first irradiation experiment 28 mutants were isolated. New mutants were further isolated in subsequent experiments including hybridizations among the induced mutants (Table II). These mutants showed that generally all features of the groundnut plant were altered. Although more than one mutant was obtained for the same character, only typical ones are given in the tables. It is interesting to note that the mutations affecting leaf and pod characters were predominant.
The range of expression in the mutated character was also large. For example, the mutant for increased plant height grew to 125-150 cm per plant while the extreme dwarf was only 10-15 cm tall (5). The mutants with increased branching produced 30-40 branches against non-branching (6) or suppressed growth of branches (7) only in others. Similarly the variation for pod reproduction ranged from 0-75 per plant and for kernel size the range was 0.15 to 1.25 gm per kernel.

In addition to the range in expression there were differential expressions of mutant characters in different seasons. For example a suppressed branch mutant developed elongated branches in other than autumn season (7) and influenced segregation studies suggesting that appropriate season should be used for genetic studies.

The breeding behavior indicated that the majority of isolated mutants were recessive in nature. The tall, large pod and long pod mutants, however, behaved as dominant types. The dominant type behavior of otherwise recessive characters in some cases were due to inherent instability of trisomies (6).

Modification of sickle shaped stipule with acuminate tip to leaflet structure, was a unique seedling mutation which expressed in F₁ when the mutant was used as a pollen parent only. F₂ segregations, however, indicated recessive nature of the foliaceous mutant character. Appearance of this recessive character in F₁, therefore, was unusual and did not conform to the expectations of Mendelian dominance (8). The differential expression in reciprocal F₁'s made this mutant a useful genetic marker detectable in crosses both as male and female parent.

Development of Trombay Groundnut varieties:

Evaluation of induced mutants and hybrid derivatives of mutants in progressive stages, resulted in the isolation of more than 19 cultures known as Trombay Groundnut (TG) varieties. These TG-varieties were and are being tested also in the national evaluation experiments.
TG-1, a large pod mutant isolated after repeated selections for increased kernel size, produced larger kernels weighing 85 gm per 100 kernels as against 50 gm of the parent (9). This variety gave an average yield of 1332 Kg/ha compared to 1205 Kg of check in the All India Coordinated Research Project trials (AICORPO) during 1969-71. Subsequently it has produced upto 5000 Kg/ha yield under irrigated conditions on the fields of cultivators. Because of this high yielding ability and large kernels of export quality, TG-1 was released by the Ministry of Agriculture, Government of India, for cultivation in 1973. Other varieties with large and extra large kernels have been isolated in crosses with other mutants.

The development of TG-varieties with large kernels has necessitated a change in the prevailing national standards (ISI standards) used for export purpose. Since large size commands premium price of about US $ 150. per ton in the world market, the export of 50,000 tons of large kernels would earn an additional income of 8-10 million US dollars.

In addition to TG-1, five other mutants also had useful characters such as early maturity (TG-2), increased pod setting (TG-3) and reduced plant height (TG-4, TG-5 and TG-6). In the yield experiments, TG-3 and TG-6 showed superior performances (9). In the AICORPO trials TG-3 yielded 18% more than others (Table III) and hence it was also recommended by the Central Ministry of Agriculture for cultivation as a high yielding variety. It might be emphasized that this variety produced significantly superior yields (4874 Kg/ha) even in the USDA experiments at Georgia in the USA during 1972-74. Yields exceeding 4000 Kg/ha have been harvested by some farmers and its seed multiplication programme is in progress.

Increased genetic variability was obtained in the crosses between mutants. Seven varieties (TG-7 to TG-13) were developed after repeated selections for five to seven generations in a cross, TG-1 x virescent. These varieties having dark
green leaves, less branches and pods without prominent veination, had more oil content (52-55%) than in the Spanish Improved parent (48-51%). TG-8, TG-9 and TG-10 had maximum oil content of 54-55% as well as superior yielding ability (10). It is significant to note that in a seed multiplication plot TG-9 yielded 4500 Kg/ha in 1976 compared to 3000 Kg in a local check.

Increases in pod yield were obtained in the newly isolated cultures viz., TG-14 to TG-19. These were also developed after inter-crossing the induced mutants. TG-14 was selected in a cross, darker green x virescent and was high yielding especially in February-May cultivation (11). TG-15 was high yielding at some locations. TG-16 with large pod character was derived after crossing TG-1 with virescent. Inspite of large kernels, it matured 15 days earlier than TG-1 and gave better recovery of kernels (Table V).

TG-17 was selected in a cross, darker green x TG-1, and had an extreme form of fastigiata plant type with only 4-5 primary branches having all flowering nodes compared to 7-8 primaries having several vegetative nodes in the control variety. Consequently the number of leaves were reduced to 50% of the normal. Since reduced vegetative growth did not influence pod number per plant, the harvest index in TG-17 was increased to 47 compared to 25 in the Spanish Improved indicating its improved production efficiency. The bold kernels of TG-17 weighing 30% more than that of the check, increased the pod yield (12). The high yielding ability of TG-17 was confirmed after growing in different locations even under unfavourable rainfall conditions (Table IV). Under irrigated conditions this variety has produced upto 5500 Kg/ha yield against 4500 Kg of check.

TG-18 variety having large pods as in TG-1 was also isolated along with TG-17 in the same cross. Being superior to TG-1 by having 5-8 days early maturity, 2-3 percent point more shelling and 10-15% higher recovery of large kernels (Table V),
increased yields were produced in the initial trials and further evaluation is in progress.

TG-19 was developed for extra large kernels with better recovery in a cross, TG-17 x TG-1. Transgressive segregation for kernel size resulted in this culture with 100 kernels weighing 120 gm compared to 85 gm in TG-1 and 60 gm in TG-17. The extra large kernels of this variety would be more useful in export trade and confectionary industry.

The Vanaspati Manufacturers' Association of India (VMA), a private organisation working in the field of oilseeds extension since 1964, took interest in TG-varieties and tested them under farmers conditions in 1974. Encouraged by the results of demonstration trials, VMA started a systematic demonstration-cum-seed multiplication programme in collaboration with BARC. Beginning with 0.8 hectare in December 1974 more than 1200 hectare were already grown under TG-varieties by the end of 1977 (Table VI). 400 tons of seed was distributed to cover more than 3000 hectare in the current year, indicating popularity of TG-varieties among the cultivators.

Summary

Mutation breeding in groundnut was initiated with a view to develop improved cultures for increasing production in India, which contributed over 33 per cent to the world groundnut production. More than 50 mutants were isolated influencing almost all features of a groundnut plant. Cytological M1 variants produced more mutants and in advanced generations.

Some mutants showed interesting genetic behaviour, while others exhibited differential expression in different seasons leading to masking of mutant characters. In addition, mutants having economically useful characters, such as large kernel size and increased yielding potential were also isolated. Using these and other mutants new Trombay Groundnut (TG) varieties were developed which had large kernels suitable for
export trade, improved oil content and increased yields. Among them TG-17 was unique for its extreme fastigiata character leading to flowering at all nodes and reduced number of vegetative branches.

Demonstrations of TG-varieties for high yielding potential on the fields of cultivators were successful. Because of increasing demand for the seed, a seed multiplication programme was initiated in 1974-75 in collaboration with a private organisation. Starting with one ton seed more than 2000 tons of seed was produced till the end of 1977 and distributed for cultivation in the current year.

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### TABLE I

**X-ray Induced Mutations In Groundnut Var. Spanish Improved (2n=40) Shown According To The Generation They First Appeared**

<table>
<thead>
<tr>
<th>$X_1$ Parents</th>
<th>$X_2$ Mutants</th>
<th>$X_3$ Parents</th>
<th>$X_3$ Mutants</th>
<th>$X_4$ Parents</th>
<th>$X_4$ Mutants</th>
<th>$X_5$ Parents</th>
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<td>Short-1</td>
<td>304</td>
<td>Non-secondary</td>
<td>304</td>
<td>Short-1A</td>
<td>304</td>
<td>Large pod</td>
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<tr>
<td>437</td>
<td>Short-2</td>
<td>304</td>
<td>Short (2n+42)</td>
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<td>Non-branching</td>
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<td>Long pod</td>
<td></td>
<td></td>
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<tr>
<td>146</td>
<td>Short-3</td>
<td>304</td>
<td>Less branching</td>
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<td>Tertiary</td>
<td>304</td>
<td>Long pod</td>
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<td>Tall</td>
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<td>Non-flowering</td>
<td>304</td>
<td>Seed lethality</td>
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<td>277</td>
<td>Cup leaflet</td>
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<td>Imparipinnate</td>
<td>984</td>
<td>Virescent</td>
<td>984</td>
<td>New virescent</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>leaf (small size)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>691</td>
<td>Darker green</td>
<td>304</td>
<td>Chlorotic</td>
<td>304</td>
<td>Xantha</td>
<td>304</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Dwarf (d&lt;sup&gt;asy&lt;/sup&gt;, d&lt;sup&gt;asy&lt;/sup&gt;)</td>
<td>646</td>
<td>Leathery leaf</td>
<td>646</td>
<td>Small pod</td>
<td>646</td>
<td>Erect branching</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Fused top</td>
<td>646</td>
<td>Leaflet pair</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>leaflet pair</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE II

Further Mutations Isolated In Groundnut

<table>
<thead>
<tr>
<th>Origin</th>
<th>First</th>
<th>Second</th>
<th>Third</th>
<th>Fourth</th>
<th>Fifth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutagen</td>
<td>1. Virescent green (vv&lt;sup&gt;v&lt;/sup&gt;g&lt;sup&gt;v&lt;/sup&gt;)</td>
<td>1. Small leaf (unstable)</td>
<td>1. Bunchy top (d&lt;sup&gt;stu&lt;/sup&gt;)</td>
<td>1. Krinkle-2 (b&lt;sup&gt;sp2&lt;/sup&gt;)</td>
<td>1. Suppressed branches</td>
</tr>
<tr>
<td></td>
<td>2. Three paired leaflets</td>
<td>2. Polymorphic leaves</td>
<td>2. Bifurcated leaflets</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4. Deep constriction</td>
<td>virescent (v&lt;sup&gt;m&lt;/sup&gt;)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5. Rusty-red seed coat</td>
<td>Minute pod (unstable)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5. Bluish red seed coat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutant</td>
<td>1. Bunchy top (d&lt;sup&gt;stu&lt;/sup&gt;)</td>
<td>Extreme dwarf</td>
<td>1. Blue seed coat</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2. Chlorina-1 (c&lt;sup&gt;1&lt;/sup&gt;c&lt;sub&gt;1&lt;/sub&gt;, c&lt;sub&gt;2&lt;/sub&gt;c&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>2. Chlorina-2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. Bifurcated leaflets</td>
<td>Virescent green (vv&lt;sup&gt;v&lt;/sup&gt;g&lt;sup&gt;v&lt;/sup&gt;)</td>
<td>2. Extra large pod (c&lt;sup&gt;1&lt;/sup&gt;c&lt;sub&gt;1&lt;/sub&gt;, c&lt;sub&gt;2&lt;/sub&gt;c&lt;sub&gt;2&lt;/sub&gt;)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4. Extreme pod constriction</td>
<td>Imperipinnate leaf (normal size)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5. Pod without beak</td>
<td>Foliaceous stipule-3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6. Pods with very prominent beak</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7. Blotched red seed coat</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The underlined are similar mutations obtained in different experiments.
TABLE III
Comparative Yield Data (Kg/ha) for TG-3, Extracted From Project Coordinator's Reports On The National Trials

<table>
<thead>
<tr>
<th></th>
<th>Ludhiana</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TMV-2</td>
<td>447</td>
<td>623</td>
<td>720</td>
<td>663</td>
<td>2023</td>
<td>1287</td>
<td>917</td>
<td>1409</td>
<td>1192</td>
<td>1216</td>
<td>571</td>
<td>993</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMV-7</td>
<td>554</td>
<td>775</td>
<td>996</td>
<td>772</td>
<td>2127</td>
<td>1277</td>
<td>1233</td>
<td>1546</td>
<td>1412</td>
<td>1400</td>
<td>519</td>
<td>1110</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-206</td>
<td>568</td>
<td>899</td>
<td>1131</td>
<td>866</td>
<td>2573</td>
<td>1538</td>
<td>1183</td>
<td>1764</td>
<td>1320</td>
<td>1356</td>
<td>278</td>
<td>985</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NG-268</td>
<td>1380</td>
<td>1009</td>
<td>1078</td>
<td>1155</td>
<td>2844</td>
<td>1492</td>
<td>1067</td>
<td>1801</td>
<td>1321</td>
<td>1420</td>
<td>414</td>
<td>1052</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J-11</td>
<td>781</td>
<td>895</td>
<td>1061</td>
<td>912</td>
<td>2365</td>
<td>1522</td>
<td>1367</td>
<td>1751</td>
<td>1536</td>
<td>1492</td>
<td>474</td>
<td>1167</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Jyoti)</td>
<td>1111</td>
<td>894</td>
<td>967</td>
<td>991</td>
<td>2956</td>
<td>1442</td>
<td>967</td>
<td>1788</td>
<td>1562</td>
<td>1482</td>
<td>484</td>
<td>1176</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG-3</td>
<td>1116</td>
<td>978</td>
<td>1128</td>
<td>1070</td>
<td>3400</td>
<td>1400</td>
<td>1083</td>
<td>1951</td>
<td>1588</td>
<td>1494</td>
<td>625</td>
<td>1226</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

|             | Sambalpur |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|-------------|-----------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| TMV-2       | 647      | 725  | 1402 | 925 | 1902     | 1642 | 1772 | 787     | 2330 | 1088 | 1402 | 1194|
| TMV-7       | 457      | 787  | 1495 | 913 | 1486     | 1652 | 1569 | 851     | 2710 | 1515 | 1693 | 1267|
| S-206       | 304      | 717  | 1160 | 727 | 1558     | 1807 | 1682 | 726     | 2369 | 1144 | 1413 | 1239|
| NG-268      | 955      | 718  | 1389 | 1021| 1750     | 2314 | 2032 | 687     | 2572 | 1198 | 1486 | 1424|
| J-11        | 1173     | 711  | 1263 | 906 | 1503     | 1727 | 1615 | 825     | 2685 | 1275 | 1593 | 1324|
| (Jyoti)     | 970      | 723  | 1382 | 991 | 1881     | 2535 | 2208 | 687     | 2612 | 1220 | 1509 | 1444|
| TG-3        | 1048     | 765  | 1509 | 1107| 1535     | 2942 | 2238 | 520     | 2870 | 1530 | 1673 | 1547|
TABLE IV

Comparative Production of TG-varieties On Farmers Fields
In Kharif 1977 (June-October)

<table>
<thead>
<tr>
<th>Region</th>
<th>Pod yield in Kg/ha</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TG-1</td>
</tr>
<tr>
<td></td>
<td>(Maharashtra)</td>
</tr>
<tr>
<td>Jalkekhurd</td>
<td>3000</td>
</tr>
<tr>
<td>Sholapur</td>
<td>1110</td>
</tr>
<tr>
<td>Kolhapur</td>
<td>5000</td>
</tr>
<tr>
<td>Pune</td>
<td>-</td>
</tr>
<tr>
<td>Ahmednagar</td>
<td>-</td>
</tr>
<tr>
<td>Sangli</td>
<td>-</td>
</tr>
<tr>
<td>Lathur</td>
<td>-</td>
</tr>
<tr>
<td>Mangrol</td>
<td>2585</td>
</tr>
<tr>
<td>Keshod</td>
<td>1845</td>
</tr>
<tr>
<td>Lathi</td>
<td>2403</td>
</tr>
<tr>
<td>Khokhadad</td>
<td>-</td>
</tr>
<tr>
<td>Hirana</td>
<td>-</td>
</tr>
<tr>
<td>Bhavanagar</td>
<td>-</td>
</tr>
<tr>
<td>Amreli</td>
<td>-</td>
</tr>
<tr>
<td>Rajkot</td>
<td>-</td>
</tr>
<tr>
<td>Dhar</td>
<td>-</td>
</tr>
<tr>
<td>Ujjain</td>
<td>-</td>
</tr>
<tr>
<td>Dewas</td>
<td>-</td>
</tr>
<tr>
<td>Khandwa</td>
<td>-</td>
</tr>
<tr>
<td>Chittorgarh</td>
<td>2500</td>
</tr>
<tr>
<td>Average</td>
<td>2635</td>
</tr>
<tr>
<td>% increase over checks</td>
<td>26</td>
</tr>
</tbody>
</table>

The yields are averages of 2-15 farms. All trials in Maharashtra and others with TG-1 were irrigated.
<table>
<thead>
<tr>
<th>Variety</th>
<th>HRS* Kernel</th>
<th>100 Kernel wt. (gm)</th>
<th>Oil %</th>
<th>Shelling %</th>
<th>Maturity days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spanish Improved (Check)</td>
<td>-</td>
<td>46-52</td>
<td>48-52</td>
<td>70-75</td>
<td>115</td>
</tr>
<tr>
<td>(Large kernels; late maturity; suitable for export)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG-1</td>
<td>28-35</td>
<td>80-90</td>
<td>46-48</td>
<td>66-72</td>
<td>135</td>
</tr>
<tr>
<td>TG-16</td>
<td>30-38</td>
<td>75-85</td>
<td>48-50</td>
<td>70-74</td>
<td>115</td>
</tr>
<tr>
<td>TG-18</td>
<td>24-33</td>
<td>85-100</td>
<td>44-47</td>
<td>70-74</td>
<td>125</td>
</tr>
<tr>
<td>TG-19A</td>
<td>20-30</td>
<td>118-130</td>
<td>-</td>
<td>68-72</td>
<td>135</td>
</tr>
<tr>
<td>(Medium kernels; early maturity; suitable for milling)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG-3</td>
<td>-</td>
<td>46-53</td>
<td>48-51</td>
<td>70-75</td>
<td>115</td>
</tr>
<tr>
<td>TG-9</td>
<td>40-45</td>
<td>50-58</td>
<td>53-56</td>
<td>70-74</td>
<td>120</td>
</tr>
<tr>
<td>TG-14</td>
<td>-</td>
<td>45-52</td>
<td>48-51</td>
<td>72-76</td>
<td>110</td>
</tr>
<tr>
<td>TG-17</td>
<td>40-45</td>
<td>55-65</td>
<td>48-50</td>
<td>69-74</td>
<td>125</td>
</tr>
</tbody>
</table>

* HRS - Trade name for export quality kernels.
**TABLE VI**

Distribution of TG-varieties

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of farmers</th>
<th>Area (ha)</th>
<th>Seed produced (pods in tonnes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1974 Dec.</td>
<td>1</td>
<td>0.8</td>
<td>1.2</td>
</tr>
<tr>
<td>1975-76</td>
<td>20</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>1976-77</td>
<td>140</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>1977-78</td>
<td>1550</td>
<td>1215</td>
<td>2500</td>
</tr>
</tbody>
</table>
MUTATION BREEDING OF DURUM WHEAT

L. ROSSI
Divisione Rad/Appl,C.S.N. Casaccia, S. Maria di Galeria, Rome, Italy.

ABSTRACT
A comprehensive programme on experimental mutagenesis was started in 1956 for both genetic research and mutation breeding at the Nuclear Center. Remarkable efforts were produced on durum wheat over the past 20 years and a lot of knowledge was gained on several aspects of this crop: radiobiology, mutagenesis, cytology and cytogenetics, genetics and breeding.
This review concern: radiogenetical studies, isolation of useful mutations, agronomic evaluation of mutant lines and use of mutations in hybridization programs. Details are given on the genetic contribution of mutagenesis to the evolution of new cultivars in durums and on the economic evaluation of the cultivars obtained by mutation breeding.
An economic return on mutation breeding of durum wheat is attempted.

1. INTRODUCTION
Durum wheat is considered a crop of semi-arid climates; it is grown mostly in Southern Europe, North America, North Africa, Near East, USSR and Argentina.
The genetical studies in this crop were almost neglected in the first half of the century: and the yield of durum was substantially lower than in bread wheat towards which the major efforts were devoted. In view of the economic importance of durum wheat in Italy and in the Mediterranean Area a programme on experimental mutagenesis in this crop was started by D'Amato and G.T. Scarascia in 1956, for both fundamental genetic research and mutation breeding.
Many efforts have been produced at the Agriculture Laboratory of Casaccia Nuclear Center, CNEN, during the past 20 years on several aspects in this crop: radiobiology, mutagenesis, cytology and cytogenetic, genetics and breeding.
In the meantime, in the world, remarkable improvement has been obtained in durum wheat: considered until 15 years ago a poor crop for poor regions, the new durum varieties are now competitive for yielding ability with the best bread wheat varieties. The variety spectrum has changed rapidly and deeply by using different breeding techniques: introduction, intervarietal-crosses, interspecific and intergeneric hybridizations, mutagenesis. All these techniques were applied at the Casaccia Center where a rapid development of new plant types was obtained with agronomic results of economic value. The paper is an attempt to evaluate the contribution of mutagenesis on the improvement of durum wheat varieties.

2. RADIOGENETICAL STUDIES
Seeds of several varieties namely: Cappelli, Russello, Grifoni, Capeiti, Aziziah, Garigliano, Appulo, Maliani 8D, LD 357, Patrizio, GA B125, Sincap have been treated both with chemical such as ethyleneimine (EI), diethyl sulphate.
(DES) and ethyl-methane-sulphonate (EMS) and physical mutagens as X-rays, fast (Nf) and thermal neutrons (Nth). The relative efficiency of the mutagens was established on the basis of M₄ spike fertility and on M₃ chlorophyll mutation frequency. In general the relative efficiency of the mutagens was as follows: EI < DES < X < NF < EMS < Nth (the range of mutation frequency being from 1 to 24%). For morphological mutations the relative efficiency was slightly different: EI < X < DES < EMS < Nth < NF (the range of mutation frequency being from 1 to 41%)

Knowledge is acquired on the mode of inheritance of induced mutations being mostly monogenic recessive. Nevertheless some mutations namely elymoid, spherococcoid and yellow green behave bifactorial when crossed with other varieties and monofactorial when crossed with the parental variety.

Dominant and monofactorial behaviour was ascertained for the short straw mutation induced in Cappelli (mutant Cp B132) and semidominant, but not definitively monofactorial, for a mutation affecting kernel size induced in Cappelli (Cp CB 2 mutant).

Several methods of irradiation (acute, chronic, recurrent) at different ontogenetic stages of the plant (such as seeds, gametes) have been applied in order to enhance the frequency of mutations and to overcome the chimaera situation. Results of durum wheat varieties subjected to chronic irradiation for all the life cycle have clearly shown the frequency of mutations is not higher as compared with those of acute seed irradiation. Repeated treatment, one or more times in different generations, has given (as expected in durum, being tetraploid species) a higher mutation frequency up to a doubling of the chlorophyll mutations when the number of exposure was repeated five times. Seed treatment as found by others, leads to a M₄ chimaeric plant and for this reason a method of gametophyte treatment have been applied. Results obtained in durum wheat and barley gametophyte irradiation have clearly shown the real possibility of overcoming chimaeric situation in the M₄ plants, being heterozigous in all the spikes for the induced mutations.

The selection of mutated plants has been carried out by applying screening methods based on visual inspection or using appropriate techniques for the identification of the expected changes on a single plant basis.

In order to establish the efficiency of both mutagens and methods of treatments the rate of chlorophyll mutations has been considered as an index of relative mutagenic efficiency. A positive correlation between the frequency of chlorophyll mutations and the frequency of morphological mutations has been demonstrated both in controlled conditions and in open field.

The distinction between qualitative and quantitative characters, even though irrelevant from the genetic point of view, is important for the plant breeder who has to choose the proper selection procedures according to the nature of the traits he is selecting for.

The induction and isolation of a wide array of mutations have been often very helpful to the plant breeder, not only for the increase of the variability, but
particularly for the change of the inheritance pattern of some characters, such as plant height which is normally under polygenic control. Mutations drastically affecting characters like culm length, kernel weight, number and length of internodes, heading time etc. have been induced in durum wheat and handled as simple inherited qualitative traits. The experimental mutagenesis has noticeably contributed to the recognition of durum as unique species. As a matter of fact a great deal of distinguishable morphological types similar to those occurring in nature has been obtained through mutagenic treatments of a limited number of durum wheat varieties. Therefore, it has been possible to argue the principles of the previous botanists who were used to classify simple morphological deviants as different species. The conclusions reached by Mackey, now shared by almost all people, points to the grouping of several tetraploid wheat species in only one species: Triticum turgidum. This assumption is well supported by the findings of the mutagenesis work. It is here sufficient to recall the mutants "spherococcoid" and "elymoid" isolated in the varieties Azizia and Capeiti. An "elymoid" mutant has been isolated even in Secale at CIMMYT; thus confirming once again the general rule of parallel variations in plants (cfr. Vavilov, 1950). These findings, reinforce the monophyletic hypothesis on the origin of the genus Triticum from one diploid ancestral species. They also indicate how to exploit the genetic variation which resides in each species. Triticale (Triticum x Secale) is just a brilliant example of how much fruitful the combination and successive engineering of different genomes can be. The exploitation of mutants with high homologous pairing due to the absence of the gene(s) Ph on the long arm of chromosome 5B can widen the scope for the introduction of alien variation in durum as well as in bread wheats.

3. ISOLATION OF POTENTIAL USEFUL MUTATIONS

In general, the M<sub>1</sub> plant raised after seed irradiation has been segregated according to the spike-progeny method; less frequently, the bulk method was applied. After the selection of mutations in M<sub>2</sub> and M<sub>3</sub> generations, great care was taken in the following two or three generations in order to make a preliminary selection of the mutants of possible value and interest for breeding purposes. Using this technique numerous useful mutations have been identified. They affect characteristics related to the improvement of durum wheat, such as culm-length, number of internodes, solid stem, size, number and disposition of leaves, lodging resistance, earliness, decreased yellow berry percentage, male sterility and resistance to diseases. The lodging susceptibility and the straw weakness of durum wheat have been a serious problem affecting the yield. Until 1964 no natural sources for culm shortening were available for the breeders in durum wheat. The induced mutants for short internode and plant height were evaluated for lodging resistance. The plant height reduction of the mutants ranged from 10 to 47% of the parental variety height, however there is no absolute corre
lation between short straw and lodging resistance since among the short straw mutants with the same culm-length marked differences have been observed. A typical case is represented by two mutant lines from Cappelli: Cp B132 and Cp B2, both 90 cm. height. The former has appreciable resistance, while the latter is as susceptible to lodging as Cappelli, whose culm-length is about 130 cm. Mutants were isolated for earliness to escape the drought, frequently occurring in the Mediterranean Area during the ripening time. The heading time, currently assumed as a good index correlated with the ripeness, has been taken as a parameter of screening.

Mutations for earliness were obtained by mutagenic treatment from the cv. Cappelli, Garigliano, Russello, Grifoni, Capeiti and LD 357. The earliness obtained in the varieties Cappelli, Garigliano and Grifoni did not exceed 2-3 days while mutants isolated in Capeiti (which is one of the most early variety) were as earlier as 8 days. In these cases the induced mutations are often associated with a shorter culm length, a certain reduction of seed set and grain size. The earlier is the mutant, the higher seems to be the probability that such a mutant be affected by other kinds of phenotypic changes, either morphological or pigmental, or by a reduction of fertility. However several mutants do occur with unchanged fertility. The early mutants were less frequent than the late ones in all the experiments of mutagenesis so far carried out in durum at Casaccia Center.

Durum wheat is a basic staple food for a great deal of world's population; hence it represents an important source of proteins. Analyses performed for protein content in a number of lines mutated for various morphological characters, as height, heading time etc., obtained from various mutagenic treatments in the cv. Capeiti, revealed that at least 18 lines, out of 173, were characterized by higher protein content ranging from 144 to 166 % of the mother variety. In spite of unfavorable association between protein content and yield a set of seven lines was identified, better than Capeiti, both for protein content (128-152 % more than the control) and yielding ability. Mutagens have been used to induce mutations for disease resistance. Among the 8 short mutant lines tested for bunt resistance (Tilletia triticoides) two mutants Cp B144 from Cappelli and Rs A1 from Russello proved to be more resistant than the controls, whereas Gr A145, a mutant from Grifoni, shows a higher susceptibility. However, the presence of material with a different degree of resistance in the original population used for mutagenesis cannot be ruled out.

Other mutations affecting morphological and physiological characters have been isolated after mutagenic treatment. They concern the absence of ligula (3 mutants from Capeiti and Castelnuovo) and the mutants with yellow green leaves (1 from Cappelli and 1 from Capeiti). Though not suitable for direct use, they were considered in cross-breeding programme for a better utilization of light by the canopies.

Awnless and smooth awns mutants, male sterile mutants have been also induced and they might be used for physiolo-
4. AGRONOMIC EVALUATION OF MUTANT LINES

a) In Italy

Having at our disposal a large stock of different mutant lines, apparently endowed with agronomical characteristics, it was possible to undertake a programme aimed at ascertaining the concrete possibility of direct use as a new variety of the best mutants identified in the preliminary trials.

Starting from the M₂ generation, large-scale field trials were first carried out in different region of Central and Southern Italy in order to evaluate the new lines in different agronomic environments [20, 26, 28]. The mutant lines were tested in comparison with mother varieties of durum wheat cultivated in Italy (Capeiti, Patrizio, Camar 7, Sincape 9, Mariestella, Ichnusa) and with foreign varieties.

Data were collected for heading and maturity time, lodging, number of spike per square meter, grain yield, test weight 1000-grain weight, yellow berry.

For all the agronomical data gathered it was clearly demonstrated that through mutations it was possible to obtain in durum wheat, lines of agronomic value giving a consistent improvement in the performance of this crop. From this extensive evaluation of mutant lines, four mutants, as a new varieties, were registered and released to the farmers.

Two lines from Cappelli (Cp B132 and Cp C48) were released in 1968 and named Castelporziano and Castelfusano respectively. In 1969 the best mutant line derived from the variety Grifoni (GR A145), was released with the name of Castel-delmonte, while in 1970 registration was requested for a line (GA B125) isolated from the variety Garigliano and it was named Castelnuovo.

b) International co-operative trials

Positive results have also been obtained from an International Programme, sponsored by FAO/IAEA [34], for the assessment of the practical value of some mutant lines in the Mediterranean and Near East Regions. Multilocation nurseries have been carried out since 1965 and 57 experimental fields, spread over 16 countries, have been settled in 4 years of trials. Each field trial included eight mutant lines, two Italian varieties (Cappelli and Capeiti) and two local varieties, chosen by each co-operator.

Useful informations on the genotype-environment interaction were achieved; the highest yields were shown by two mutant lines (GA 125 and GR A145) and by Capeiti variety. From these co-operative trials we could surely infer that for the typical areas of durum wheat: North Africa, Middle East and generally, in the Mediterranean Region, early and lodging-resistant lines are required. Also, it seemed that local varieties could be easily equalled or outyielded by new introductions as shown by the performance of the induced mutant lines.

5. USE OF MUTATIONS IN HYBRIDIZATION PROGRAMS

Crosses between mutants and mother variety, between mutants for the same (or different) trait(s), coming from the sa-
me (or different) variety(s); between mutants and other varieties, have been performed at Casaccia Nuclear Center over the past 15 years [27, 35]. The objectives of this program are as follows:
- transfer of beneficial mutations in other genotypes;
- combination in the same genotype of two or more mutations affecting the same character or different characters;
- analysis of the genetical behaviour of the mutations;
- development of new varieties of durum wheat.

Mutants from cultivars Cappelli, Garigliano, Capeiti, Azizia, Grifoni, Russello, Sincapa have been largely used in a cross breeding program aiming at obtaining genotypes possessing mutations for reduced plant height and earliness. In general it has been found a good combining ability of the mutants for the more remarkable agronomic traits, namely yielding potential, quality and disease resistance. The genetic effect of mutations for short straw has been shown to be additive either for mutations induced in different varieties, or for different mutations isolated in the same variety as in the case of the mutants Cp B132 and Cp C48, both induced in the cv. Cappelli. In the segregating generations plants shorter of either parents have been selected. These new types possess both mutations in homozygote condition. Furthermore, such condition implies other modification to other characters such as earliness, grain quality and yield. The double homozygotes are more productive and slightly later than Cappelli and than the either parent mutants Castelporziano and Castelfusano. Minor mutations (mutations with less phenotypic expression) often accompany major mutations: plant height as in this case. The presence of the minor mutations can be detected in derivatives of the backcrosses, thus permitting the isolation of the main mutation which in so doing, can be cleaned of all other aberrations and other deleterious minor effects. The minor mutations are sometimes positive in that they can modify the expression of the major mutation or alternatively can increase the variability of other quantitative characters. The mutant Cp B144 from Cappelli has good grain quality (i.e. high 1000-kernel weight, low yellow-berry), other than lower stature as compared with the mother variety. These traits have been particularly useful for the improvement of the quality. In fact from the cross of the mutant Cp B144 with mexican lines carrying Norin-10 dwarfing genes, good lines have been selected and released as new outstanding cultivars with the names CRESO and MIDA. These varieties add to the well-known good agronomic characteristics of the mexican material (short straw, high spike fertility, photoinensitivity, high yielding capacity, etc. etc.) good technological quality of the Cp B144 (high 1000-grain weight, good test weight and good quality of the pasta) [36, 37, 38].

A complete list of the durum varieties obtained at the Casaccia Nuclear Center, from direct mutants of from crosses with mutants, is shown in the table 1.

6. CONTRIBUTION OF THE MUTAGENESIS TO THE EVOLUTION OF NEW CULTIVARS IN DURUM WHEAT

To this purpose is worth showing a synthetic picture dealing with the present panorama of durum wheat varieties.
cultivated in Italy /39/. The Italian durum varieties can be divided in three groups according to their genetic development and agronomic characteristics.

In the first group, Cappelli and Capeiti are included; the last one is still now the most cultivated in Italy.

In the second group there are some varieties with specific improvement in comparison with Capeiti: Trinakria, Maristella, Hymera, Castelporziano, Raineri, Polesine, Montanari, Granato, Eliodoro, Campomoro and Sincape 9. Their yielding ability and adaptation are not significantly higher if compared with Capeiti. Some of them are early flowering and early ripening and can escape to drought. Trinakria is the best one for protein content and even for technological quality. Another one, Appulo, exhibits usually good agronomic performances in Southern Italy and is largely cultivated, though it is susceptible to lodging, rusts and mildew. Otherwise all these varieties do not utilise properly the very good agroclimatic conditions because of their lodging and disease susceptibility. The very new high yielding varieties are included in the third group. Valsacco, Valnova, Valgiorgio, Valfiore, Creso Valnera, Valselva and Tito. All of them, except Tito, are semidwarfs types with Norin-10 genes. Tito is the only one durum variety which is competitive in yielding ability and lodging resistance with the semidwarf types. It was selected from a cross between Lakota and Castelporziano, which is a short straw radioinduced mutant from Cappelli. Tito represents a valid alternative to the "monoculture" of Norin-10 types in the very good agroclimatic conditions. Compared with Capeiti, all of them are late flowering and late ripening, some show remarkable resistances to stem rust, leaf rust and mildew. Yellowberry can be easily avoided by proper applications of nitrogen fertilizers, which are "a must" for these varieties. They are recommended for very fertile soils with good availability of water and fertilizers. In such conditions their yielding ability is competitive with bread wheats. In the table II, the grain yields of bread wheats and durums (Capeiti, Creso and Tito) are compared in more than 80 trials performed in Central Italy in the period 1970 - 1976 /40/. The yielding ability in new durums is not less than in new bread wheats and this behaviour is largely confirmed by the data in the farms. In durum wheat grain filling and ripening period are longer and more elaborated than in bread wheats and this fact could represent an uncertain factor mainly in yielding stability. On the other hand disease resistance (mainly stem rust and mildew) are easily incorporated in these tetraploids. In 1977 an heavy attack of stripe rust, unusual in Italy, damaged the majority of the Italian bread wheats without serious attack on many durum varieties.

The genetic progress obtained in yielding ability is clearly illustrated in the fig. 1. The data are referred to the new lines selected at the C.S.N. Casaccia (Rome) and included in the comparative trials, for each year, from 1961 to 1976. Capeiti is considered the test variety and the differences in yield from Capeiti of the "field average", the "10 % best lines average" and the "20 % best lines average", are reported.
From 1961 to 1968 the number of the lines included every year in the trials was less than one hundred, later it was more than two hundred.

Until 1968 the "field average" was significantly less than Capeiti and no appreciable improvement was obtained. From 1969 to 1974 the improvements of the new lines appear clearly very rapid and significant.

The utilization (fig. 2) of new short straw lines (from mutants and mainly from Norin-10 genes) and the incorporation of rust and mildew resistances, were the determinant factors of this rapid genetic improvement.

To evaluate the contribution of the mutagenesis, apart from the cultivars Creso and Tito, is useful to look at the fig. 3 where the lines, developed at C.S.N. Casaccia from 1961 to 1977, each year for the first time in agronomic trials, are distinguished in four groups according to their genetic origin:

- direct mutant lines
- lines coming from crosses with mutants
- lines carrying Norin-10 dwarfing genes
- lines coming from crosses involving neither Norin-10 nor mutants.

It can be noticed that in the beginning the genetic material undergoing field trial was represented almost exclusively by pure mutants, as a result of the big effort devoted to the induction of mutations.

During the period 1965 to 1967 corresponding to the development of a big cross-breeding program involving mutants and varieties, appear in agronomic trials some lines coming from crosses of local varieties available at that time. However, being their natural variability extremely reduced it was not possible to obtain significant genetic improvements. Starting from 1969 the availability of lines coming from crosses of mutants and Mexican material (Norin - 10) produced a big jump in terms of yield. From now onwards the number of direct mutants decreases dramatically, whilst the derivatives of the crosses between mutants and varieties keep on a good level, despite the big impact of the Norin-10 short straw material.

The success of the selections carrying genes from Norin-10 can be given for granted in highly fertile soils occurring in the area surrounding Casaccia Nuclear Center, but in zones sub-arid typical of Mediterranean Area it is likely to infer a better adaptability of the mutants over Norin-10 material. This pattern comes up frequently in trials carried out in South Italy, particularly in Sicily where mutants for earliness induced in Capeiti seem to be very promising and they are largely used in crosses.

Trying to understand the main reasons of the remarkable success of durum wheat programme, we must underline the following points:"primitiveness" of the species, great efforts on radiogenetical and fundamental studies, integration of different breeding techniques, adoption of valid methodology of isolation and evaluation of the new derivatives. In the table III is reported a synthesis of 17 years (1961 - 1977) of agronomical evaluation and selection of the new lines obtained in the Casaccia Center. By using this agronomical procedure it was possible to discard every year about 70-80% of the lines in trials (98.4% of the initial number in only 3 years),
therefore many new lines could be tested and properly evaluated.

7. ECONOMIC EVALUATION OF THE CULTIVARS OBTAINED BY MUTATION BREEDING

Among the varieties released (Castelporziano, Castelfusano, Casteldelmonte, Castelnuovo, Creso, Mida, Tito, Giano, Febo and Augusto) Creso is the most important from an agronomic and economic point of view. It was released in 1974 and more than 350,000 Ha were cultivated in Italy in 1977-78. The mean yield over all the Italian surface was estimated in 1976 as 3.16 tons/Ha against 1.75 tons/Ha for Capeiti and 2.12 tons/Ha for Patrizio. The higher yields of Creso are explained even by the better agroclimatic condition compared with Capeiti and Patrizio. Underestimating a benefit of 0.9 tons/Ha, the total benefit for Italy in 1978 due to Creso amounts to 315,000 tons, equivalent to $ 7, millions. The surface cultivated with this new variety is in rapid expansion as we can realise looking at the data on the certified seeds.

The percentages on total durum certified seeds are reported according to the relative contributions:

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Capeiti</td>
<td>38.7</td>
<td>33.3</td>
<td>37.1</td>
<td>35.9</td>
<td>25.4</td>
</tr>
<tr>
<td>Patrizio</td>
<td>36.8</td>
<td>35.1</td>
<td>33.4</td>
<td>31.0</td>
<td>21.3</td>
</tr>
<tr>
<td>Creso</td>
<td>-</td>
<td>-</td>
<td>1.1</td>
<td>5.0</td>
<td>16.5</td>
</tr>
</tbody>
</table>

The data for 1977 were not available but it is reasonable to estimate a proportional increase of Creso and decrease of Capeiti and Patrizio.

Trying a balance we can estimate the cost of mutation breeding on durum wheat, comprehensive of the studies on radiobiology, mutagenesis, cytology and cytogenetics, genetics and breeding, carried out in the last 15 years in the C.S.N. Casaccia about 3.5 millions $. Obviously some of these data are approximative but they represent globally the real situation.

Acknowledgments

Thanks are due to all people of CNEN RAD/APPL Division who have contributed to set up this work.
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TABLE 1. - Mutants and varieties released by the Casaccia Nuclear Center, CNEN.

<table>
<thead>
<tr>
<th>Varieties or mutants</th>
<th>Parents</th>
<th>Mutagenic treatment</th>
<th>Main improved attributes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castelfusano</td>
<td>Cappelli</td>
<td>Thermal neutrons $1.05 \times 10^{13}$/cm$^2$</td>
<td>yield and lodging resistance</td>
</tr>
<tr>
<td>Castelporziano</td>
<td>Cappelli</td>
<td>Thermal neutrons $8.38 \times 10^{12}$/cm$^2$</td>
<td>&quot;</td>
</tr>
<tr>
<td>Cp B 144</td>
<td>Cappelli</td>
<td>X-rays 20 Kr</td>
<td>grain properties</td>
</tr>
<tr>
<td>Casteldelmonte</td>
<td>Grifoni</td>
<td>Fast neutrons 100 reps</td>
<td>yield and lodging resistance</td>
</tr>
<tr>
<td>Castelnuovo</td>
<td>Garigliano</td>
<td>X-rays 15 Kr</td>
<td>&quot;</td>
</tr>
<tr>
<td>Creso</td>
<td>Cp B 144 x (Yt54N10-B) Cp$^2$ x 53$^2$/Tc$^2$</td>
<td></td>
<td>very high yielding ability</td>
</tr>
<tr>
<td>Mida</td>
<td>Cp B 144 x (Yt54N10-B) Cp$^2$ x 63$^2$/Tc$^2$</td>
<td></td>
<td>&quot;</td>
</tr>
<tr>
<td>Tito</td>
<td>Castelporziano x Lakota</td>
<td></td>
<td>&quot;</td>
</tr>
<tr>
<td>Augusto</td>
<td>(Castelporziano x Lakota) x Casteldelmonte</td>
<td></td>
<td>&quot;</td>
</tr>
</tbody>
</table>
Table 2. Yield and hectolitric weight of bread and durum wheats obtained in 80 agronomic trials conducted in central Italy in the last 8 years

<table>
<thead>
<tr>
<th></th>
<th>Bread wheats</th>
<th>Durum wheats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Irnerio or Flaminio</td>
<td>Capeiti</td>
</tr>
<tr>
<td>Mean yield (q/Ha)</td>
<td>54.01</td>
<td>40.56</td>
</tr>
<tr>
<td>Mean hectolitric weight (kg/hl)</td>
<td>77.65</td>
<td>81.29</td>
</tr>
</tbody>
</table>

(After Bozzini, Mosconi, Rossi, 1978)
Tab. 3. - Synthesis of 17 years (1961-1977) of agronomical evaluation and selection of new lines of durum wheat obtained in the Casaccia Center.

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of lines tested</th>
<th>Number of trials</th>
<th>Number of replications</th>
<th>Plot size m²</th>
<th>Selected lines (% of tested)</th>
<th>Selected lines (% of initial No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>13.500</td>
<td>1</td>
<td>2</td>
<td>1-2</td>
<td>21.6</td>
<td>21.6</td>
</tr>
<tr>
<td>2nd</td>
<td>2.922</td>
<td>1</td>
<td>4</td>
<td>6-8</td>
<td>30.8</td>
<td>6.7</td>
</tr>
<tr>
<td>3rd</td>
<td>900</td>
<td>2-4</td>
<td>4</td>
<td>10-12</td>
<td>24.6</td>
<td>1.6</td>
</tr>
<tr>
<td>4th</td>
<td>221</td>
<td>3-5</td>
<td>4</td>
<td>10-12</td>
<td>26.7</td>
<td>0.4</td>
</tr>
<tr>
<td>5th</td>
<td>59</td>
<td>5-10</td>
<td>4</td>
<td>10-12</td>
<td>40.0</td>
<td>0.18</td>
</tr>
</tbody>
</table>
FIG. 1 - DIFFERENCES IN YIELD (Capeiti - O) OF DURUM LINES TESTED AND SELECTED (10% - 20%)
FIG. 2 - DIFFERENCES IN HEIGHT (Capeiti - 0) OF DURUM LINES TESTED AND SELECTED (10% - 30%)
FIG. 3 - TOTAL NUMBER OF LINES EACH YEAR FOR THE FIRST TIME IN AGRONOMIC TRIALS AND % OF COMPOSITION FROM THE FOUR CATEGORIES ESTABLISHED
MUTATION BREEDING IN ROOT AND TUBER CROPS

A.M. van Harten.
Department of Plant Breeding, Agricultural University, Wageningen, the Netherlands.

Abstract

Mutation Breeding in Root and Tuber Crops.

Proceeded by a few general considerations about problems and results of mutation breeding in vegetatively propagated plants a review is given of the results of mutation breeding programs up to now in the different (tropical) root and tuber crops (cassava, sweet potato, yam, potato and others).

1. General Introduction.

Root and tuber crops belong to the group that are vegetatively propagated for commerce, although most of them are also more or less capable of cross-breeding. They are generally heterozygous and often of polyploid or aneuploid nature. In contrast to many other vegetatively propagated crops there is not the drawback of a long juvenile phase.

Application of cross-breeding methods in these crops often leads to a display of considerable genetic variation, because of the mentioned high heterozygosity, which makes it difficult to maintain the general characteristics of the recurrent parent and to offer also a base for selection of cultivars which are superior in one or a few characters.

Selection of spontaneously arisen mutants (sports) has led to an impressive number of new cultivars of vegetatively propagated crops (Leon [1]). Right from the beginning of deliberate mutation breeding activities it was realized that vegetatively propagated crops are particularly well suited for this purpose. However, in contrast to the very encouraging results obtained with vegetatively propagated ornamentals and, to a lesser extent with fruit trees, the outcome of work with induced mutations in root and tuber crops has been limited up to now.

The main explanation for this may be that in ornamentals "novelty" is one of the main features whereas at the same time selection for better types is often much easier. The occurrence of accompanying mutations, as is commonly the case, has less important consequences than for food crops; e.g. a profusely flowering ornamental which is somewhat later or has a slightly changed leafshape does not lose its acceptability from a buyers point of view, but increased yield, accompanied by bitter taste or bad tuber colour in food crops diminishes their attractiveness. Even a better taste will not always find acceptance because of the conservatism of the consumers. An additional point with respect to food crops is that most important characters like
yield, protein content, adaptability, etc. are inherited polygenically, which makes their detection after mutagenic treatment more difficult.

As induced mutations supplement natural variation, their role should be assessed together. Whether the spectrum of induced mutations as a group is comparable with or inferior to that of spontaneous mutations has been subject of much discussion. In this context it may be considered that many spontaneous mutants with selective disadvantages have never been taken into account.

The discovery of useful spontaneous variation mostly occurs by accident and so-called negative sports are simply removed. In mutation breeding programmes, on the other hand, it is common to register all aberrations and to express the number of potentially useful mutations as a percentage of the overall mutation frequency. Therefore a direct comparison seems not a correct approach. Moreover, spontaneous mutations result from sets of conditions which include very much variation in conditions of climate, soil etc. and which may stretch out over an impressive number of generations.

The proper way of deciding whether mutation breeding techniques or other breeding methods should be used should be based on economics and is determined by the genetic nature of the character(s) concerned, the breeding system of the species and the availability of a discriminative selection method in order to recognize the desired genotype.

Mutations mainly go from dominant to recessive. From this point of view the high degree of heterozygosity in vegetatively propagated crops facilitates their detection. On the other hand it is clear that genetic characters which are governed by one or more dominant genes cannot normally be obtained by mutation breeding methods, unless it is possible to work with very large numbers of plants (e.g. adventitious bud techniques) and to apply a very efficient screening method. When this is not possible either different starting material for mutation programmes, if available, should be looked for or cross breeding with other, probably distant relatives should be considered. However, even in the latter case mutation breeding might be useful in order to perfect the result of the crossing program.

Most mutations studied up to now concern characters with simple inheritance. As small mutagenic steps occur more often than big steps (Gaul [2]) and the frequency of usable induced variants from micro-mutations seems to be much higher than for macro-mutations (Gregory [3]), the importance of mutations for characters with continuous variation, should be stressed here. In addition, the viability of plants carrying micro-mutations is often considerably better than that of plants with macro-mutations.

The problem of chimerism occurring after mutation breeding has been discussed before at many occasions (see e.g. the Manual on Mutation Breeding of the IAEA, 2nd ed., 1977). It may suffice here to say that chimerism occurs after a mutagenic event in one cell of a multicellular organism. In vegetatively propagated plants such mutated cells either are lost again, or, via mericlinal chimeras,
are becoming periclinal chimeras, because of the layered structure of e.g. young shoot apices. Real mutated 'sectors' do not occur in vegetatively propagated crops and solid mutated plants (so-called homohistonts) are an exception, unless special measures are taken. There is a rapid increase of the application of in-vivo as well as in-vitro methods of adventitious bud formation. It appears that by such methods problems of chimerism can be solved in practically all cases.

The in-vitro techniques themselves have brought to our attention that small explants often may display a much higher spontaneous mutability than is the case with complete plants. This subject needs further attention. In order to explain the phenomenon one should refer to the role of a controlling and stabilizing mechanism, operating in complete plants.

With respect to a choice for either physical or chemical mutagens, there exists not much evidence that chemicals offer considerably better perspectives for mutation breeding, notably in vegetative propagated crops, because of the size of most treated material, poor penetration and problems of reproducibility. In-vitro methods offer better prospects. The hope that chemicals may work on particular genes is but extremely small in the light of our present knowledge that genes are linear sequences of only four nucleotides. On the other hand it is not impossible that some specificity may be found in later, secondary steps by the occurrence of specific "sieves" (Auerbach [4,5]).

As for vegetatively propagated crops, there have been many doubtful reports in the past concerning the induction of high frequencies of mutations. Such results often referred to only one or a very few generations. Moreover, there is no doubt that in some publications effects of radiation damage and true genetic effects have been mixed up. The finding of Kukimura and Takenata [6] that in potato radiation damage is also transmitted to V′M2 stresses the need for continued cycles of observation.

In all mutation experiments with field crops, more than with for example greenhouse crops, several generations should be studied as the effect of year or field may be very considerable. As to screening for possibly induced resistance in the field (e.g. for Phytophthora infestans in potato), in some years such resistances cannot be studied at all.

In the following review on mutations in (mainly tropical) root and tuber crops not all literature has been discussed. For a full account see Broertjes and Van Harten [7].

**Cassava** (*Manihot esculenta*).

From the fact that cassava and its wild relatives show very much genetic variation it could be concluded that there is no urgent need for mutation breeding work in order to increase genetic variability.
The low number of publications concerning mutation breeding probably reflects this point of view.

It is, on the other hand, not unlikely that improvement of an already accepted cultivar or outstanding crossing product can be achieved more easily by mutation breeding than by cross breeding, even when cultivars are used which show a high degree of fertility. As is known, cassava is a tetraploid (2n=4x=36) which makes cross breeding not an easy task. When considering possibilities of mutation breeding, the pattern of inheritance of the characters in which one is interested, and the possibility of easy and early detection should be considered.

The first mutation experiment known to me, described by Moh [8], mainly dealt with determining radiosensitivity. By applying several mutagenic agents, Vasudevan et al [9], not only obtained morphological mutants but also types with increased starch content and a decreased hydro cyanide content.

Abraham [10] and Moh and Alan [11] mentioned the induction of mutants, some of which looked promising from the point of view of yield increase.

In addition to irradiation of vegetative material Moh and Alan also irradiated cassava pollen with dosages varying from 2-50 krad of γ-rays, the main advantage being that in this way no chimeras are produced. For stem cuttings at least two vegetative cycles are necessary to obtain solid (or periclinal chimeric?) mutations.

Nayar [12] applied acute and chronic γ-rays as well as EMS to complete plants or to stem cuttings of 15-20 cm., with dormant buds of the recommended Indian cultivar M4 and a few other promising cultivars. Acute γ-rays were applied in dosages up to 5 krad. Chronic irradiation was continued during 8 months with dose rates up to 30 R/hr. The EMS treatment (by means of wrapping vegetative buds with cotton dots) yielded no mutations. The radiation treatment gave several mutations which were mainly of a morphological nature. Chronic irradiation led to increased HCN content with increasing dose rates. Several mutants such as an interesting type with uniform cylindrical tubers, were still under observation. The author refers to differences in sensitivity between diploid and tetraploid (or octoploid?) clones, the tetraploids being more radiosensitive and their yield being lower.

Moh [13] reported that after γ-irradiation of young stem cuttings of cv. Japanese some will plants were found with a lower HCN concentration. It is clear that screening in following vegetative generations must be performed in order to confirm such results.

The efficiency of mutation breeding in cassava may be increased when a reliable adventitious bud technique would be available. Kartha et al [14] described such an in-vitro method.

It is clear that sprouting of buds from irradiated cuttings is increasingly inhibited with increasing dosages. From the available publications it appears that, depending on the cultivar, acute irradiation of noded stem cuttings, with up to 4 or 5 krad should be advocated, the LD50 being below 3 krad.
The most promising approach seems to be to start from high yielding, virus resistant cultivars in which for example tuber shape or flesh colour should be improved in order to increase consumers' appeal.

**Sweet potato (Ipomoea batatas).**

Sweet potato, a hexaploid (2n=6x=90) is a difficult crop from a breeding point of view. In many cases it shows a low level of flowering and seed set, incompatibility and sterility barriers.

The occurrence of spontaneous mutations has been described at many occasions. Hernandez et al. [15] mentioned spontaneous mutation rates for tuber skin colour of up to 2.9%. Yen [16] estimated the change from white or cream to orange or purple at roughly 1:1000. Such figures, however, should be treated with care as they may reflect the age of the crop as well as the number of individuals studied (Leon [1]).

Studies on induced mutations were published as early as 1935 (Miller [17]). Sweet potato appears to be a very promising crop for mutation breeding (see e.g. Gustafsson and Gadd [18]). Broertjes and Van Harten [7] mentioned a total of 25 publications in this respect. Most studies refer to irradiation with X- or γ-rays of rooted or unrooted stem cuttings of various ages. At some occasions leaves, roots or tubers were treated and occasionally also seeds.

In an early experiment Cheng [19] irradiated tubers with 1-5 kR X-rays. Later the use of dosages up to 50 kR has been reported, but in most cases irradiations do not exceed 10-15 kR. In some instances neutron irradiation was applied (Love [20,21]) and as far as known in one case the chemical EI (ethylene-imine) was used (Kukimura and Takenata [6]). Repeated treatment with Phosphorus-32 was reported by Sakai [22].

Mutations of practical interest which have been reported so far include a wide scala of characters such as early maturity (Cheng [19]), higher yield and higher starch content (e.g. Cheng [19], Poole [23], Poole and Tanaka [24], Sakai [22]), tuber skin and tuber flesh colour (Hernandez et al. [25], Kukimura [26], Sakai [22]), stem diameter (Sakai [22]), sugar content (Kukimura and Takenata [6]) and cold tolerance (Miu [27]).

It appears that mutation breeding may lead to improvement of several valuable characteristics in sweet potato and to the release of new cultivars after a short time, provided that this work is carried out in a systematic way, starting from the best cultivars available and that one looks for realistic objectives.

Finally it may be observed that adventitious bud production in sweet potato is possible (Wilson [28]), which may considerably facilitate mutation breeding work.
Yams (*Dioscorea* spp.).

Although yams are of considerable importance, notably in West Africa, breeding research in yams has been rather limited and this is also the case for mutation breeding. The increasing importance of the production of diosgenin may stimulate future agricultural research.

It appears that ample room for improvement can be found in selecting in and combining of the available genetic potential but cross-breeding is seriously hampered by poor flowering and seed set. In this respect mutation breeding should mainly concentrate on further improving cultivars which have already found a certain degree of acceptance.

High yielding mutants were obtained by Abraham [10] after treating tubers with γ-rays. Koo and Cuevas [29] concluded from their experiments with irradiation of aerial tubers with 2 krad of γ-rays, that removal of the first shoots and allowing new shoots to be formed leads to (apparently) solid mutant shoots in vM2.

Pal and Sharma [30] studied irradiated *Dioscorea* tubers and resulting mutants for three consecutive generations after a treatment with 15 krad X-rays. A marked increase in diosgenin was observed in vM2 and vM3.

Yams can be multiplied by the use of detached leaves, which may make mutation breeding more attractive.

Potato (*Solanum tuberosum*).

Potato undoubtedly is the most investigated tuber crop. An impressive amount of literature concerning mutation breeding work in potato is available (for full references see Van Harten [31]). Only a short summary of the most important experiments and conclusions can be given here.

Commercial cultivars arisen from spontaneous bud variations have been described since 1857 (Cramer [32]). In the U.S.A. between 15 and 35% of the potatoes grown for certified seed production have been reported to come from bud sports (Krantz [33], Heiken et al. [34]). In other countries like the Netherlands and the U.K. this percentage has never been above 1%. Most of the useful sports refer to changes in structure (e.g. russeting) or colour of the tuber skin.

The first X-ray experiment in potato with reported increase in yield and in tuber size was performed in the early twenties by Jacobson [35]. The first definite proof of the genetic nature of mutations was given by Asseyeva and Blagovidova [36]. Heiken [37] was the first worker who studied mutation breeding techniques in potato emphasizing the practical applicability. This important contribution provided valuable information on different mutation types and their frequencies, but Heiken did not find mutants of direct practical value.
In following years many useful mutations have been reported like those for earliness, shorter stolon-length, resistance to various diseases, shallow tuber eyes, dayneutrality, induced starch content, tuber yield, tuberskin colour, etc. Many of those findings unfortunately, refer to small scale experiments, which often were restricted to only one or a few generations. In addition, useful mutations may be accompanied by unfavourable effects. A well-known example in this respect is the occurrence of cracks in tubers in a promising mutant with shortened stolons (Roer [38]).

All kinds of mutagenic agents, physical as well as chemical, have been used. Mostly tubers or tuber parts like single tuber-eyes have been irradiated, mainly with X- or γ-rays, the optimal dose being around 2-3 krad. At present also irradiation of cuttings is performed. Cuttings have the advantage of being more uniformous starting material. New in-vitro techniques are becoming available, which increases the possibility to overcome the problems of chimerism, encountered up to now.

As far as I am aware, despite considerable efforts only one new induced cultivar (cv. Konkei: released in Japan in 1973) with improved tuber skin has been registered up to now, whereas some positive, but as yet unconfirmed results should have been obtained in the USSR.

Several studies mentioned above have sufficiently demonstrated that there are no reasons to question that mutation breeding in potato could lead to positive results, provided again that the proper starting material, correct procedures and realistic goals are chosen.

Other root and tuber crops.

Only very few publications concerning mutation work in many minor root and tuber crops are available. Mutants of practical interest have not been reported up to now. Because of the low level of breeding of most of those crops in general simple breeding methods like clonal selection still may lead to considerable progress and there seems to be little reason to expect that work with induced mutations in the near future would lead to practical results in a quicker or less laborious way. This does not rule out that the crops within this group are in most cases well suited for mutation breeding, especially if good adventitious bud techniques were available.

Concluding remarks.

Broertjes and Van Harten [7] present a list of 146 commercial mutants of vegetatively propagated crops. Only one, concerning potato, belonged to the category of root and tuber crops (see footnote).

* (N.b. This list was closed on December 1st, 1977. At present (September, 1978) over 200 of such mutants are on record in the literature).
This sufficiently demonstrates that not much has been achieved yet in root and tuber crops.

As mentioned before there are sufficient indications that in certain situations mutation breeding can lead to positive results. In this respect I fully support the opinion, expressed at many occasions (see e.g. Nybom [39]) that mutation breeding is mainly a complementary tool or method to other breeding methods. The main decisive factor for its use is an economical one, i.e. in which way can the breeder reach his objectives in the cheapest or quickest way.

When comparing results of different methods it should be kept in mind that it is not justified to look to the outcome without taking into account the labour involved and the amount of starting material. It is indeed strange to observe that sometimes miracles are expected from very small scale irradiation experiments or, on the other hand, mutation breeding methods are condemned because such small experiments give results that are inferior to large-scale cross breeding programs.

There are situations in which mutation breeding is to be preferred, e.g. the improvement of a good cultivar in one or a few characters which are governed by recessive genes. Sometimes there is no alternative to mutation methods at all, e.g. when the material involved is sterile or unsuited for crosses. When considering the possibilities, it should be kept in mind that, on the other hand, nobody benefits from mutation breeding work with unrealistic objectives or an inadequate set up.

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CONTRIBUTED PAPERS
Efforts for crop improvement through the use of radiation induced mutation have started in Egypt more than twenty years. Among the more promising results was the induction of high protein lines of field bean (Vicia faba L.), field bean mutants resistant to Callosobruchus maculatus and a gossypol-free mutant of cotton.

Efforts conducted in Egypt through the last twenty years in the field of the utilization of induced mutations for improving different crops are presented and discussed. It is concluded that mutation work in Egypt needs to be better organized and directed toward specific objectives to improve its economy.

Research work on radiation effects on Egyptian agricultural plants was, initially, mainly designed to collect basic information about germination [34, 74], seedling growth [9], cytological effects [44, 48, 66] and characters of $M_1$ plants [28, 106, 110, 134].

Different mutagenic agents were used for treating seeds of various plants in these studies. Gamma-rays emitted by radium were used on ornamental species of Matthiola, Viola and Delphinium [74]; Cesium-137 on cotton [64, 134] and kenaf (Hibiscus cannabinus) [110]; Cobalt-60 on various crops [9], wheat [106], barley [59], rice [60], maize [10], sorghum [48], cotton [125], berseem or Egyptian clover [28]; peanut [75], field bean [11], lentil [12, 52], chickpea [12], helba or fenugreek [12], lupina [12], sesame [33, 90], flax [38], kenaf [110], tomato [76], onion [129], squash [113], okra (Hibiscus cannabinus) [3, 115], Pisum sativum [89] and Dianthus.

X-rays were used for maize [66, 112] and Pisum sativum [127]. radioactive phosphorus ($^{32}$P) was used as a mutagen agent on cotton-seed [52].

Several chemical mutagens were utilized for treating the seeds of different crops: EMS on rice [60], Vicia faba [84], wheat [102], Pisum sativum [127], and Salvia splendens [88]; DES and EA on Vicia faba [71], NMU [111] and ET [117] on barley. Colchicine was also used for the ornamental plants Cosmos sulphureus [77] and marigold Tagetes erecta [94].

Mutation breeding programmes have acquainted Egyptian plant breeders to fast and reliable screening method for protein [17, 19] and oil content [33, 41, 42, 43] of the seed.

It may be appropriate to summarize the research work according to groups of plant species.
1. **Research work on cereals and grains:**

1.1 **Wheat:**

The total cropping area in Egypt is about 11,630,000 feddans* [53], wheat acreage is about 13,940,000 feddans yielding about 20,330,000 metric tons [53] which is not sufficient to cover local demands. Research work on induced mutations may be classified into three directions:

1.1.1 **Breeding for disease resistance:** Trials aiming to induce resistant mutants of wheat against specific races of stem rust (*Puccinia graminis tritici*), leaf rust (*P. recondita*) and stripe rust (*P. striiformis*) were carried out [7, 8] by exposing seeds of local varieties to various doses of Co-gamma rays ranging from 10 Krad to 20 Krad. Authors obtained 73 resistant mutants out of 2,770,080 M₀ plants [7]. Most of these mutants carried some deviations in other plant and yield characteristics in comparison with their mother varieties. None of the more resistant mutants obtained have so far been officially approved for either direct use or for cross breeding by our Ministry of Agriculture.

1.1.2 **Breeding for modified plant characters:** Other breeding programmes aimed to modify plant characters such as culm length [27, 67, 103, 104, 122, 124], flowering time [27, 104, 106], tillering [27, 67], awn length [27] no. of florets per spike [67], number of leaves [67]. These experiments included the utilization of either Co-gamma ray doses ranging from 5 Krad to 35 Krad or EMS [102, 103] concentration 0.12 M (1000 cm⁻³ per 250 seeds for 6 hours) for treating seeds of most of our local varieties in addition to a limited number of imported lines or varieties [101, 102, 104, 121]. Sometimes without convincing reasons for introducing such newly imported and "non-adapted" genotypes in a programme for inducing mutations.

Following treatment of seeds of the local variety Giza 155, containing different moisture levels (5.5 - 18.9%) with 60 Co-gamma ray doses between 10 and 35 krad, it was found [108, 124] that means of culm length, spike length, number of spikelets and kernels per spike were generally reduced in M₀ and M₁ generations especially in highest doses at water content below 11.0%. Variability was increased in both generations, more apparent in M₁ than in M₂ [123]. Morphological mutants have been obtained in M₂ generations such as erectoid, compactoid and speltoid [67]. Their frequencies were affected by delayed planting in M₁ and the dose used.

By treating seeds of a local variety with EMS (0.12 M - 1000 ml/250 seeds for 6 hours), means of characters were significantly shifted in some experiments [27, 101] and not affected in other [102, 103, 104] but variability was generally increased in M₂ and M₃ generations.

It has been reported that some mutants [67, 132] with practical value have been isolated. However, there is no evidence for any of them being used directly or in hybridization programmes.

1.1.3 **Breeding for improving yield components and quality or technological characters:** Much work has focused on yield components, e.g. tillering capacity [106], spike length [103, 104], number of spikelets [67, 108, 122, 123, 124], number of grains per spike [67, 108, 124], weight of 100-kernels [67, 104, 108, 122, 124], grain yield per plant or

* 1 feddan = 4200 $m^2$. 

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per spike [27, 101, 132] and spike sterility [67]. Protein content of kernels [27, 30, 121] as well as technological characters [121] such as sedimentsation value, ash, fat and crude gluten content of seed have likewise received some interest in mutation breeding experiments.

Seed irradiation with doses ranging from 5 Krad to 35 Krad of Co-gamma rays or treating them with EMS (0.12 M - 1000 ml per 250 seeds for 6 hours) resulted mostly in negative changes [101, 106, 107] of characters such as number of grains per ear, seed set/spikelets and grain yield per plant in M₀ generation. Means of some of these characters were not affected [104], but for variability in characters such as weight of 100-kernels, heritability estimates were greater in mutagen treated - EMS, 0.12 M or 25 Krad of Co-gamma ray - populations in all traits than in untreated population.

Some investigators [103] found that irradiation was superior to EMS in increasing genetic variability in M₀ generation and in one investigation it was concluded to postpone selection in mutagen derived populations at least until M₁ generation where the variability was greater than that in M₂ [108].

A comparison between the effect of mutagen treatments (25 Krad of gamma rays or EMS - 0.12 M) and hybridization on variability, showed [104] that hybridization created about twice as much variability as mutagen treatments.

In one experiment [108] eight mutant types were isolated in M₁ plants through M₀, M₂, and M₃ generation. Large spike mutants with high tillering capacity were selected in M₀ [67]. Other mutants concerning yield components were isolated in M₃ [122]. However, none of these mutant types were later on utilised in Egypt.

The same holds for mutants concerning quality and technological characters. The seed treatment of some local and also an imported line with either 25 Kr of Co-gamma rays or EMS (0.12 M) caused in M₁ lines significant increases in protein percentage, crude gluten, free fatty acids and sedimentsation. But ash and fat percentages were decreased [121].

Studies were also performed on the adaptability of some mutants in comparison with Mexican wheats at two locations in Egypt [109]. The results may still need more confirmation.

1.2 Maize:

The planting area of maize covers about 16% of the Egyptian cropping area. Maize production (around 2781000 metric tons) [53] is not sufficient to cover local demands. Mutation breeding on maize is relatively rare due to the (cross pollinating nature of this crop plants) and the availability of a very large amount of genetic variability. However, Co-gamma rays (from 0.5 to 10 krad) or x-rays (1 to 5kR), were utilized for irradiating seeds of either inbred lines [10, 40, 43, 66, 78, 112, 120] to be self pollinated for obtaining inbred lines with modified characters or to cross them with other treated or untreated lines or open pollinated populations [78] or single and double crosses [66, 120]. The frequency of aberrations (either morphological or cytological) increased with increasing dose [120].

Different genotypes differed in their radiosensitivity [66]. However, the radiosensitivity of the P₁ and P₂ of the single and the double cross hybrids was dependent upon their parental inbred lines. The double cross hybrid was dependent upon their parental inbred lines. The double cross hybrid was least affected by irradiation. Means of plant characters, yield and oil content of kernels [43] of inbred lines treated with mutagenic agents were slightly affected but variability was increased in M₁, M₂, and M₃ generations. Some abnormal types were isolated in M₀ generation after irradiating seeds of inbred lines. However, corn breeders did use any mutants.
1.3 Rice:

The cultivated area with rice is about 1052000 feddans producing about 2423000 metric tons [53] which is usually sufficient to cover local demands and a certain quantity for export.

Main objectives for mutation breeding work [35, 58, 59, 60] on rice were to obtain dwarf or short culm lodging resistance, early flowering, resistance to disease (mainly blast) improving kernel shape and technological characters, and to improve nutritional value (protein content and quality). Mutagens used for seed treatment were Co-gamma rays (5, 40 krad) fast neutrons (doses equivalent to 1.2, 1.6 and 2 Krad, applied at the IAEA Laboratory Seibersdorf) and EMS (0.2%, 0.4% and 0.6%).

An academic [73] investigation showed different varietal responses to various doses and the increasing sterility of M₃ plants as the gamma ray dose increased up to 40 krad. Attempts to improve kernel-protein (DBC-protein) led to significant shifts in the mean of the M₃ generation and an increase in variability of this character [35, 59]. Mutation breeding for improving other characters in the "introduced" American good kernel-low tillering yielded 17 mutant lines [59] with higher tillering variety, Blue Belle but unfortunately without retaining other good characteristics of the parent variety. Four mutant lines resistant to blast disease were also obtained from the local (blast-susceptible) variety Nahda [59]. Three of the blast-resistant lines showed good accordance with other characteristics of the parent variety Nahda. These results may support the view of the author that it would be better to utilize the best local varieties (or lines) and not the newly imported (non-adapted) ones for mutation breeding. It has also been reported [59] that half of the blast resistant lines were induced by 20 krad, one fourth by 12 krad of gamma-rays and the rest by the fast neutron treatment equivalent to 1.6 krad. These observations could lead to conclude that gamma rays are recommendable for inducing such mutants. However, up to the present, none of the mentioned mutant lines has been directly utilized or used in cross breeding.

1.4 Barley:

The average annual cultivated area with barley is around 100000 feddans producing about 118000 tons [53].

Mutation experiments with barley in Egypt have studied the effect of mutagenic agents in M₂ [67, 105, 111, 117], generations attempted to increase genetic variability by inducing mutations [95, 96, 97, 98, 130] and included crosses between mutant lines and their original mother varieties [2, 86].

Mutagenic agents used in seed treatment were Co-gamma rays [67, 70, 95, 96, 97, 98, 111, 130], (10, 30 krad), n-Nitroso-n-Methyl-Urethane (NMU), 0.02%; 1000 cc/250 seeds, and combination [117] between gamma rays and Ethylenimine (EI, concentration of 2.9 or 5.8 M) or nitrogen mustard (NM, concentration of 0.12 M).

Observations were taken on cytological aberrations, stainability of pollen grains, chlorophyll defects, heading date, plant height, number of tillers and leaves per plant, number of spikes/plant, number of sterile florets/spike, spike length and density, number of kernels per main spike, kernel yield/plant [111], weight of 1000 grains [67], morphological abnormalities and mutants. Stainability of pollen grains and fertility of florets were usually decreased by increasing the dose (or concentration) of the mutagen agent used.

Significant shifts in means of most of the investigated characters were recorded [97, 117]. Variability for characters was increased by mutagen treatments, particularly by chemical mutagens [111]. Cytological and morphological abnormalities [105] were higher in M₃ than in M₄.

The frequency of chlorophyll mutations increased by increasing the dose, however, the highest rate of early flowering mutants were obtained by the 15 krad treatment [95, 98]. These early flowering mutants - (185) - were classified into 19 different groups [96, 130] according to ear characters and subjected to further evaluations [97]. Two-rowed large
kerneled mutant type were isolated from the original six-rowed mother variety [105]. Other mutants were erectoids, dwarfs, short straw or high tillering.

Crosses between the early flowering mutants [2] and their original mother variety (Giza-116) lead the investigators to conclude that "induced mutants in cross breeding may open new possibilities in improving crop plants through heterosis phenomenon". Genetic variation in segregating families (crossing three gamma ray induced early flowering mutants and their original mother variety Giza-117 was found to be mainly due to dominance and additive effects [86]. But none of the mutants was utilized yet commercially or in breeding programmes of the official authorities responsible for improving this crop.

1.5 Sorghum:

The planting area of sorghum is around 489000 feddans yielding about 775000 tons [53].

The only published work on the effect of seed irradiation of sorghum with Co-gamma rays (10 - 60 kR) noted effects on germination, mature plant height, survival, kind and extent of meiotic aberrations and somatic mutations in two local varieties [48]. Cytological abnormalities and reciprocal translocations were noted in M1 and M2 generations.

Besides significant shifts in the mean values of the other characters, it seems that this work did not continue further.

2. Research work on fiber crops:

2.1 Cotton:

Cotton is the most important fiber crop (and also oil crop, see 3.1) in Egypt. The average planted area of cotton is 1346000 feddans producing about 382000 tons of fiber and 663000 tons of seed [53].

Some of the Egyptian commercial varieties of cotton such as "Menoufi" include a wide range of variation in seed, plant and lint characters [6]. Nevertheless, studies for making use of mutagenic agents for increasing genetic variability in cotton have been started more than twenty years ago [4].

Usually for seed treatment of local varieties, varietal hybrids (vh) and back crosses between vh and one of the parent varieties [118], mutagenic agents used in Egypt included: Ultra-violet [52], x-rays [52, 135] gamma-rays emitted by 32P (seed treatment with a known activity solution) [125], 137Cs (555 - 3999 R) [50, 52, 64, 134] and Co [50, 52, 54, 62, 63, 64, 65, 131] (0.5 - 50 kR); EMS [68] (100 - 5000 ppm), or a combined treatment of 1250 ppm EMS + 10 Kr of Co-gamma rays [118]. 32P was also used in activities of 5, [49] - 65 microcuries per seed [52, 79].

Characters investigated in various generations included: Germination, leaf characters, plant height, number and date of flowering, position of first sympodial branch, sterility and pollen grains, number of fruiting branches, branchlessness, date of first boll, number of mature bolls, boll weight, cotton yield, seed set, seed yield, seed indices, glandlessness, ginning out-turn, lint yield or hair yield, staple length in micronaire readings, halo length, fiber strength, fiber finess, half fall, lea product and abnormalities [49, 50, 54, 62, 64, 68, 79, 125, 131, 134, 135, 136].

Objectives of work using mutagen agents on cotton were and still are:

- to induce mutations such as, short vegetation period (early flowering);
- the economic branchlessness suitable for mechanical harvesting;
- glandlessness or gossypol-free (i.e., edible cotton-seed-protein);
- fiber yield and quality (spinning quality).

Differences in the response of various varieties to mutagen agents have been noted.
Mutagen agents caused significant shifts in means of some characters. Stimulation in plant height by low doses of gamma rays, inhibition on yield characters especially by high doses was noticed in M_1 generation [125]. Some researchers found positive effects in some seed characteristics [54], for boll weight [54] and in spinning tests [50, 118]. Variation was increased in plant height and halo length [136]. High variability has been noted among homozygous lines in both after EMS and gamma ray treatments [118]. Mutations could be induced by gamma rays also in quantitative characters [50]. However, mutations affecting the whole appearance of the plant appeared only at high doses, i.e., 20 - 50 KRad of Co-gamma rays [131].

The following mutations of potential value have been induced in Egyptian varieties of cotton:

(a) A glandless mutant [49] strain "Bahtim-110" was obtained after treating seeds of variety Giza-45 with 40 microcuries of ^32P per seed for 48 hours. Glandless means gossypol-free, i.e., cotton-seed-protein can be utilised for infant nutrition [100]. This mutant had acceptable fiber and yarn qualities [61] but lower yield than the parent Giza-45. The character was found to be partially dominant, depending on one pair of genes [50, 116, 126, 133] and it was easily transferred in crosses with most of our varieties [5, 61].

(b) Leaf mutations such as, broad leaves, wrinkled leaves and leathery leaf [62, 136].

(c) Dwarfs and semi-dwarfs [79].

(d) Floral mutations [79]: i.e., first sympodium at higher node (obtained by 20 - 30 KRad ^32P).

(e) Branchless type [62, 63, 137] which is suitable for mechanical harvesting.

(f) Smooth boll [126].

(g) Superior halo length and improved fiber qualities (EMS mutant) [68].

(h) Improved resistance for fusarium wilt [5].

Neither the glandless mutant line Bahtim-10 nor the other above mentioned mutants so far have been used in commercial production.

2.2 Flax:

The annual average area planted with flax in Egypt is about 54000 feddans yielding about 139000 tons of straw and above 27000 tons of seeds [53]. Local varieties usually serve the dual purpose, i.e., oil flax for painting, minor industries and linen fibers for textile.

Only a few reports have been published on mutation breeding of flax [36, 38, 42]. Co-gamma ray doses ranging from 0.5 to 100 Krad have been used for irradiating seeds of two local varieties to improve this crop by induced mutations in characters such as plant height, flowering and flowering time, number of branches, capsules per plant, seed yield per plant, oil content in seed, oil quality and fiber properties.

Published results [36, 38] show in M_1 significant shifts in means of some characters such as oil seed content (increased by about 17%) and unsaturation of oil. Variability for most of characters was increased by radiation.

Our study is still in progress. Unpublished mutants selected in M_1 which bred true in M_1 and M_2 concern flower colour, size and petal position. The possibility of pleiotropic effects of these mutations upon economically important characters is under investigation.

2.3 Kenaf:

Kenaf (Hibiscus cannabinus L.) - an important jute substitute but slightly weaker, coarser and more brittle than jute - is mainly used for
weaving bags and cordage. The kenaf planted area is very limited, probably around few hundred feddans.

Gamma ray doses ranging from 200 r to 30 Kr (emitted either by $^{137}$Cs or $^{60}$Co) have been used for irradiating seed [110]. Means of characters such as plant height, number of nodes to first flower, ribbon yield to first flower were shifted in $M_1$ generation (increased by the $^{60}$Co-gamma ray doses of 100 - 1000 R but no effect for similar doses emitted by $^{137}$Cs). Fertility was reduced by medium and high doses. Number of days from planting to blooming was not affected in $M_1$. Coefficient of variation values in most of investigated characters were increased by irradiation. A mutant in the colour of flower was isolated in $M_2$. This work did not continue.

3. Research work on oil crops:

3.1 Cotton:

Cotton is the main fiber and edible oil crop in Egypt. However, mutation work done on this crop did not include any investigations on oil content (and quality) of cotton seed.

3.2 Flax:

See Section (2.2.)

3.3 Sesame:

Sesame ($Sesamum indicum$ L.) planted area usually amounts to 33000 feddans yielding about 17000 tons of seeds [53].

Mutation breeding of sesame has been started with irradiating seeds of the two major varieties Giza-23 (white seeded) and Giza-24 (buff-brown) to Co-gamma ray doses of 1,5 and 20 krad [33, 41, 90, 91, 92]. Plant and yield characters were studied in $M_1$ and $M_2$ generations [90].

Oil content of the seed was screened by traditional as well as recent methods including NME [33, 41].

Some significant shifts in means of plant and yield characters were observed in irradiated populations in $M_1$ and $M_2$ generation. Variability was usually increased by radiation. Thirty mutant lines have been selected [91, 119] in $M_2$ and confirmed in $M_4$. These mutations were obtained from the (buff-brown-seed) variety Giza-24 but not from the other (white-seed) variety which reflect a view for higher mutability of the former over the latter variety. Twenty six of these mutations were found in $M_4$ progenies of the 20 krad treatment. The rest (four) mutants were induced by the 5 krad [92, 119]. General features for these induced mutants are:

(a) Seed yield per plant for most of mutants was significantly higher over the original mother variety.

(b) Higher seed yielding mutants possess higher number of capsules per plant.

(c) Wider fruiting zone than mother variety.

(d) Oil yield per plant was higher.

(e) Delayed flowering was obtained in one of these mutants.

(f) Variability of most - not all - characters is still higher in $M_4$ than respective variability in mother variety.

These mutants are in $M_4$ generation for further evaluation.

3.4 Peanuts:

The average annual cultivated area in Egypt of this crop is around 32000 feddans yielding about 28000 tons [53]. One third of this amount is usually devoted for exportation.
The effect of irradiating seeds of two local and two imported varieties of peanuts with Co-gamma rays (5 - 25 kR) was studied on plant and yield characters in $M_1$ and $M_2$ generations.

Gamma ray seed irradiation caused in $M_1$ decreases in stand at harvest time, main stem height, spreading distance, hay weight, yield per plant, number of pods per plant, number of loculi per pod and seed weight [75]. Increases in number of branches and empty loculi percent were also noticed.

Plants exceeding in some characters the ranges of the control were observed. This work did not continue.

4. Research work on forage crops:

4.1 Egyptian clover:

Egyptian clover or berseem (Trifolium alexandrinum L.) covers about 25% of the total cropping area in Egypt [53]. However, work and reports on mutation breeding for improving this crop are very rare [1, 28]. The effect of irradiating seeds of the two major local varieties were studied. The main objective was to improve seed setting, to increase yield (cutting) and to modify flowering time. Results concerning the variability and mean shifts in some of the investigated characters in $M_1$ generation were obtained. This work is in programme.

5. Research work on legumes:

Due to narrow variations exhibited within Egyptian varieties of legumes [1], efforts for increasing variability through mutagen agents have been made on field bean, lentil, chickpea, helba (Trigonella foenum graecum) lupins and peas.

5.1 Field bean:

Field bean (Vicia faba) occupies about 281000 feddans yielding about 239000 tons [53] being not sufficient to cover local demands.

60 Co-gamma ray doses of 0.5 - 18 Krad or EMS concentrations of 0.025, 0.050 and 0.100% or EA concentrations of 0.05, 0.15, 0.25 and 0.5% or EMS concentrations of 0.015, 0.020, 0.025 and 0.030 M or combination between gamma-rays and chemical mutagens were used [71, 84, 87]. Colchicine was also applied in concentrations of 0.1, 0.3, 0.5 and 0.7% for seeds or terminal bud [83].

General aims for improving this crop through mutation breeding may be classified as:

Seeds of local varieties were utilized in these studies [45]. Twenty five mutants with 9.2% to 34.0% increase in their protein content over mother varieties were obtained in $M_2$ and bred true till $M_6$ [17, 23, 24]. The two mother varieties showed different mutabilities as far as seed protein content is concerned. The same for the doses of gamma-rays used. Correlations between protein content of these mutants and other characters of plant and yield were investigated [20, 24]. Some high protein lines were sent to official authorities for testing. Many characters of plant and yield components of the high protein lines were subjected to variable amounts of change [37]. It seemed that it was possible to break the negative correlation usually found between seed yield and seed protein content [23, 85].

High protein lines were obtained also by EMS treatments [85]. Amino acid spectrum was changed but not in favour of limiting essential amino acids, i.e. sulphur containing amino acids.

5.1.2 Flowering time:

Early flowering mutants [82] and also late ones were selected in $M_2$ and subsequent generations. However, in later generations the earliness of lateness was statistically insignificant [11, 47, 71].
5.1.3 Pest and disease resistance:

Efforts have been made to breed through mutation induction field beans resistant to chocolate spot disease caused by \emph{(Botrytis fabae)} and to rust \emph{(Uromyces fabae)}. Some achievements in this respect were reported [7, 8, 11, 45, 71]. However, no resistant mutant was directly propagated or included in further breeding programmes.

Breeding field bean mutants resistant to the insect pest \emph{Callosobruchus maculatus} P. (Diptera) yielded promising results [14, 15, 26]. It was found that white-seed-coat mutants were resistant whereas the brown or violet ones were susceptible, a trend which was found to be valid even in lines obtained by conventional breeding [39]. Another attempt was made to explain the resistance to this insect on chemical basis [16, 18, 31, 32]. None of these mutants have been used yet commercially.

5.1.4 Yield and yield components:

Mutants with higher number of seeds per pod were obtained [21]. Slightly increased yield was noted in \( M_2 \) or later generations [82, 85].

5.1.5 Other plant characters:

Mutations concerning fertility [83], long stem [83], dwarfs [82], plant height [81], number of branches [8, 72], coloured seed coat [82], leaf shape and texture [71], colour of hilum [83], were reported. However, none of them were utilized in breeding programmes.

5.2 Lentils:

Average planted area of lentils in Egypt is about 58000 feddans yielding about 39000 tons [53].

\(^{60}\text{Co}-\text{gamma rays were used to increase variability in some characters of this plant [12, 51, 114]. However, only preliminary results of mutation breeding work on this crop have been reported. Increases in variability of some characters by irradiation (doses below 11 kr) may encourage the continuation of a programme for improving this crop through induced mutations.}

5.3 Helba:

Helba or fenugreek \emph{(Trigonella foenum graecum)} covers about 38000 feddans [53] of our planted area. However, effort for improving this crop through induced mutations is almost nil. Increased variability in \( M_1 \) generation after \(^{60}\text{Co}-\text{gamma ray seed irradiation with doses ranging from 0.5 to 5 kr} has been reported [12].

5.4 Chickpea:

Chickpea occupies about 6000 feddans of Egyptian cultivated area producing about 4000 tons [53]. The plant received some interest for improving through mutation breeding.

Studies on the effect of \(^{60}\text{Co}-\text{gamma rays} (0.5 to 5 kr) on variability of some plant and yield characters were carried out [12]. A three-seeded pod mutant has been found [22, 25] a pod type which is not found in any Egyptian variety. Original mother variety contains one-seeded pods and two-seeded pods (in a ration of 1:1.1) and no three-seeded pods at all. The irradiated population \( (M_1) \) showed a ratio of 1:1.6, \( M_2 \) showed 1:1.3:0.1 (three-seeded pods) and \( M_3 \) ratio was 1:1.1:0.2. Investigations on this character are still in progress.

Chickpea mutants rich in sulphur-containing amino acids have been reported [13]. Studies on protein mutations are in progress[29].

5.5 Lupin:

Average annual area planted with this crop is around 8000 feddans yielding about 5000 tons of seeds [53].
No work on improving this crop through induced mutations was done except for an experiment aiming to study the effects of Co-gamma ray doses ranging from 0.5 to 5 Kr on the variability of some characters of this crop in M1 generation [12].

5.6 Peas:

Peas (Pisum sativum) has been subject of several mutation induction experiments using gamma rays or EMS [57, 89, 127, 128]. Results are more of academic interest and no mutant has been used commercially.

6. Research work on vegetable crops:

Average annual area devoted for vegetable crops in Egypt is around 895000 feddans yielding about 6520000 tons [53]. However, mutation breeding for improving species belonging to this group received minor interest. This may probably be due to the fact that a major part of seeds or other parts used for propagating the vegetable plant is imported.

Okra (Hibiscus esculentus L.) [3, 115], Onion (Allium cepa L.) [129], Tomatoes (Lycopersicon esculentum) [76] and Squash (Cucurbita pepo) [113] are the vegetable species so far used in mutation experiments. However, induction of useful mutation through mutagenic agents did not exceed investigations in M2 generations.*

7. Research work on ornamental flowers:

Ornamental flowers such as Mattiola [74], Viola [74], Delphinium [74], Cosmos sulphureus [77], Marigold (Tagetes erecta L.) [94], Salvia splendens [88] and Carnations (Dianthus caryophyllus) [55, 56] received some attention in studies on effects of mutagenic agents such as gamma rays [55, 56, 74] EMS [88] and colchicine [77, 94]. Little progress has been achieved towards practical goals.

**CONCLUSIONS**

It must be concluded that mutation breeding work in Egypt yielded very few important achievements.

Mutation breeding projects have helped to gain knowledge about genetics and physiology of important crop plant characters and have promoted the utilization of appropriate (fast) screening methods for protein for oil.

This survey on induced mutation experiments in the last two decades in Egypt may be useful as an example for participants in this Seminar. Although, the former and current work reviewed involved considerable efforts the gain might have been more fruitful if mutation breeding programmes would have been organized towards clear breeding goals. This would have saved labour and money in a country like Egypt with its limited financial resources. This recommendation may also be valid for any of the developing countries and the rich ones as well.

It would also appear useful to co-operate in National, Regional, African or International work for the improvement of different agricultural crops.

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* S.A. El-Sayed selected late blight resistant mutants of tomato. They were tested in M1 and found to contain higher amounts of phytoalexin. In: Induced Mutations Against Plant Diseases, IAEA Vienna (1978), 265.
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MUTATION BREEDING FOR RESISTANCE TO THE COCOA
SWOLLEN SHOOT VIRUS: PRELIMINARY STUDIES
VICTORIA APPIAH
DEPARTMENT OF BIOLOGY, FOOD AND AGRICULTURE
GHANA ATOMIC ENERGY COMMISSION

ABSTRACT

Attempt is being made to supplement conventional
breeding with induced mutation breeding to control the swollen
shoot disease of cocoa in Ghana. In the preliminary studies
doses of below 5Krs were found not to be lethal to the
Amelonado seeds. Further work is in progress.

INTRODUCTION

Cocoa has been the major foreign exchange earner for Ghana.
It contributes 65% of the national foreign exchange (Buxton, 1973).
Ghana's position as the world's leading exporter of cocoa is
currently being threatened by fast decline in her productive capacity.
Between 1961 and 1965, Ghana produced 35% of the world's total cocoa
but by 1974 she was producing 26% (Anon. 1974).

Among the major factors contributing to the decline are the cocoa
swollen shoot virus (CSSV) disease insect (capsids) attacks and smuggling
across the borders. The use of insecticides against the capsids and rigid
security measures by the government against smugglers are projected to
neutralise these factors.

The swollen shoot disease is essentially West African, having been
found in the Ivory Coast (Albert 1946) Togo (Kenten & Legg 1971) Sierra
Leone (Attafuah, Blencowe and Brunt 1963) and in Nigeria (Murray 1946).
This disease was first reported in Ghana in 1936 when a farmer noticed
swellings on branches in the Eastern Region (Steven 1936). The disease
was thought to be caused by poor soil conditions but later Postnette (1940)
showed the causal organism was virus. There are many strains of the virus,
and each of these produce different symptoms of the disease found on the stem,
leaf and pod. The new Juabeng and Bisa strains produce large swellings, the
Nkawkaw strain a few swellings and Kpeve strain no swellings at all. Affected leaves are crinkled, malformed and have yellow pattern or vein bonding. There is premature shedding of leaves. Affected pods are small, almost spherical, having a smooth surface with reddish patches. The flat seeds produced never contain the virus (Clerk 1974).

Serious attempts to control the problem by removing diseased trees and paying compensation to the farmers was started in 1941, when it was believed that the disease could be erradiacted by this means (Kenton and Legg 1971). By 1961 Quartey Papafio (1961) considered that the disease was well under control except in the Eastern Region where continuous vigilance was required to check rapid spread. Legg (1975) estimated a mean annual revenue lost from removing diseased trees over a period of 28 years to £3.65 million. Although some control was achieved by removing trees sporadic outbreaks of the disease do occur.

Control of the disease through conventional breeding was started soon after the disease was reported (Postnette 1942). After screening several hybrid types a level of resistance was found among Nanay and Iquitos populations (Postnette and Todd, 1951, Dale 1957). Some Amazonian varieties were introduced to the farmers on basis of the rate of growth and yield. Later on crosses between these and Amelonado was found to be superior so these hybrids were introduced into the breeding for resistance and tolerance to swollen shoot programme and hybrids were distributed to farmers. These hybrids were later found to be susceptible to infection with the virus (Legg & Kenten 1970). Legg and Lackwood (1976b) thought the level of resistance could be increased by replacement of the Amelonado parent with an alternate clone. This programme is still continuing and more resistant hybrids obtained will be released to farmers.

Thus the breeding for resistance to the swollen shoot disease in Ghana has up-to-date been conventional methods. There is the need for the introduction of new variability in the host. Induced mutation provides variability which could help in improving the resistance of the crop to the pathogen. In view of the seriousness of the problem and the importance of cocoa to the national economy, the Ghana Atomic Energy Commission has initiated an induced mutation breeding programme aimed at producing resistant lines.
MATERIALS AND METHODS

Before the main breeding programme could be started it was considered necessary to study the effect of gamma-radiation on the seeds and to determine the effective dose. Batches of fresh beans of Amelonado cocoa obtained from Tafo Cocoa Research Institute, were irradiated at 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 2.5, 8, and 10 krs, using a Cobalt-60 source at the Ghana Atomic Energy Commission. Trails were conducted on seedbeds as well as in seed-boxes. The seedlings were observed for thirty days during which germination records were taken daily.

RESULTS AND DISCUSSION

Germination was normal up to 1 kr dose. Then the germination percentage started to decrease (Table 1). The germination at 2 Kr was 59% while that of 5 Kr was 9% but none of them survived. The radicals appeared and then died. The doses of 5 Kr and above was lethal to the seeds. The LD50 for Amelonado cocoa seeds used was 2 and 5 Kr. Opeke and Jacobs (1972) made a similar observations.

The first leaves of the irradiated seed were found to be malformed. The malformations became severe with the increase in radiation dose. The damage of the seedlings from 1 Kr and below was low and almost negligible in 0.2 Kr irradiated seedlings. Opeke and Jacobs (1972) observed leaf anomalies such as forking of leaves, variegation and many leaf margins of cocoa seedlings from irradiated seeds. Since these were not observed on the seedlings from non-irradiated seeds it was concluded that the malformations were due to irradiation. The seedlings recovered from radiation damage after the third flush leaf when the subsequent leaves appered normal.

The preliminary study will not be limited to Amelonado variety alone. Other varieties currently being used in breeding work including hybrids being planted by farmers will be studied for their response to irradiation and then subsequently used in the main programme.

The breeding work is aimed at obtaining either tolerance or high level of resistance. Since there are a number of strains of the virus, resistance or tolerance to the most virulent strain will go a long way in helping to solve the problem.

Although conventional breeding for resistance to the swollen shoot has gone on for a number of years a highly resistant cultivar
has still not been obtained and the disease is still economically important. It is hoped that induced mutation by irradiation will increase the genetic variability of the crop and enhance the search for a highly resistant mutant to the cocoa swollen shoot virus.

**TABLE 1**

Percentage germination of irradiated cocoa seeds 30 days after sowing

<table>
<thead>
<tr>
<th>DOSE (a) (Krs)</th>
<th>% GERMINATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>94</td>
</tr>
<tr>
<td>0.4</td>
<td>94</td>
</tr>
<tr>
<td>0.6</td>
<td>92</td>
</tr>
<tr>
<td>0.8</td>
<td>80</td>
</tr>
<tr>
<td>1.0</td>
<td>76</td>
</tr>
<tr>
<td>1.5</td>
<td>69</td>
</tr>
<tr>
<td>2.0</td>
<td>59</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>96</td>
</tr>
</tbody>
</table>

\[ a) \quad \text{Source of irradiation is Cobalt - 60 at G.A.E.C.}\]

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An attempt to adapt the American soyabean variety Clark-63 to short-day conditions using gamma-rays and ethyl methanesulphonate (EMS).

C.K. Bulungu
Kings College Budo,
P.O. Box 7121,
KAMPALA, Uganda.

ABSTRACT

A preliminary study was made in an attempt to adapt the American soyabean variety Clark-63 to the short-day conditions prevailing near the equator using low, medium or high doses of gamma-rays and ethyl methanesulphonate (EMS).

Dry seeds of Clark-63 at 11.6% moisture were irradiated with gamma-ray doses ranging from 5 to 45 krad at Seibersdorf, Austria. The irradiated seeds were sown together with other seeds treated with EMS doses ranging from 0.5 to 2.3% EMS at the Makerere University Farm at Kabanyolo (0° 28'N; 32° 27'E; 1160 metres above sea level).

In the M1, the mutagenic efficiency of gamma-rays and EMS was compared at comparable M0 growth reduction. Low, medium and high mutagenic doses were studied. Chlorophyll mutants and mutants with increased plant height were scored; only increased plant height was used as the indicator mutation for positive macro-mutants.

EMS was more efficient than gamma-rays for the induction of chlorophyll mutations and positive macro-mutations.

Although chlorophyll mutation rate increased with increase of gamma-ray dose, the frequency of positive macro-mutations was highest at the medium dose.

By using plant height as the indicator mutation, it was possible to select mutants with increased: (a) vegetative period, (b) days to maturity, (c) number of branches, (d) main stem node number, and (e) seed yield.

INTRODUCTION

The origin and early history of the soyabean crop are unknown. From the review by Probst and Judd it is a native of eastern Asia (25). On the basis of historical, geographical and taxonomic evidence, it was suggested that the soyabean first emerged in eastern North China (16).
Soyabean is not adapted for production in short-day regions of the world. In general, varieties adapted for production in the United States of America where most breeding work in soyabean has been carried out, flower too early to make adequate growth for producing satisfactory seed yields when planted in short-day regions (13). The American variety Clark-63, for example, reaches early bloom stage only after four weeks when grown in Uganda whereas in America soyabean varieties flower after six to eight weeks (31,14).

Genetic types are available which require long days to reach early bloom stage when grown at low latitudes. Unfortunately these genotypes have poor agronomic qualities and Hartwig has recommended them to be used as non-recurrent parents in backcrossing programmes with agronomically desirable cultivars to develop productive cultivars adapted for production in tropical regions (12,13).

It is apparent that this breeding procedure requires a minimum of five generations whereas through mutation induction, the identification of a positive macro-mutant in an agronomically desirable cultivar requires only two generations.

The study reported in this paper was carried out in an attempt to use experimental mutagenesis to adapt the American soyabean variety Clark-63 for production in short-day conditions in Uganda, and to compare the mutagenic efficiency of gamma rays and ethyl methanesulphonate at low, medium and high doses. The American soyabean variety Clark-63 was chosen as the experimental material for the following reasons:

1. When grown in Uganda the plant of Clark-63 is very short and almost branchless which was considered to be advantageous in screening for macro-mutants with increased plant height. A non branching plant type was considered to be ideal for comparing low, medium and high doses of mutagens, since in a branching soyabean type increased branching associated with low plant populations at high doses would reduce the mutation rate at these doses as a result of increased diplontic selection (11).

2. Clark-63 being the recommended soyabean variety for production at high density in Uganda (15), it was anticipated that the induction of mutants with improvements in its shortcomings notably: (a) too short vegetative period, (b) short plant height and (c) too low main-stem node number, would be of direct value.
MATERIALS AND METHODS

Dry seeds of the American soyabean variety Clark-63 were used in the present investigation.

Batches of seed at 11.6% moisture were irradiated with 5, 10, 15, 20, 30, 35, 38, 40, 42 or 45 krad of gamma rays at 2000 seeds per dose at Seibersdorf, Austria on 24th February 1976. The irradiation treatments were performed in four replicates of 500 seeds per dose. Immediately after irradiation, the seeds were put sealed in thick polythene bags. They were then sent by air from Vienna to Entebbe, Uganda.

Other batches of seed from the same lot were treated with 0, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5, 1.8, 2.0 or 2.3% EMS for 3 hours at 24°C on 26th March, 1976. The treatment solution was applied at a rate of 2 ml per seed. Each treatment consisted of 2000 seeds but the treatment was performed in four replicates of 500 seeds per concentration. After treatment the seeds were post-washed in running tap water for 5 hours in four replicates as during treatment.

M₁ generation

Gamma-ray irradiated seeds and EMS - treated seeds were sown together in a complete randomised block design with four replicates on 26th March 1976 at the Makerere University Farm Kabanyolo. The experiment was grown in isolation from other soyabean varieties to ensure that any changes observed in later generations are of mutational origin. The spacing used was 60 cm between rows and approximately 5 cm within rows. In order to estimate the effect of dose on seedling growth, epicotyl length was measured on 25 seedlings selected randomly from each replicate, 14 days following sowing. Epicotyl length was used instead of seedling height because earlier experiments had shown that for all practical purposes the need to convert measurements to net growth as has been suggested (5) can be avoided and in addition more meaningful dose/response curves are obtained when epicotyl length is used than when seedling height is used.

The M₁ plants were harvested in bulk, each treatment separately.

M₂ generation

During the second rains of 1976, the mutagenicity of gamma-rays and EMS was compared at comparable M₁ growth reduction as measured by epicotyl length. Doses causing about 10, 50 and more than 75% M₁ growth reduction were considered to be low, medium and high respectively. The doses were 5 and 10 krad of gamma-rays
versus 1.1 and 2.0% EMS. The 45 krad-dose of gamma-rays, representing a high dose, was included for comparison with the 5 and 10 krad doses, the last two representing low and medium doses respectively. Unfortunately there was no EMS dose equivalent to the 45 krad of gamma-rays in the range studied in the present experiment.

On 5th October 1976, the M_2 was sown in a complete randomised block design with four replicates. The spacing used was 60 cm between rows and 10 cm within rows. The high M_1 sterility at the 45 krad dose restricted the number of seeds to be studied in the M_2 generation to 6600 seeds per treatment.

Plant height was the only selection criterion used in screening the M_2 for positive yield macro-mutants. In soyabean yield is known to be positively correlated with plant height (1, 20, 34).

Mutation rate was scored as number of mutants per 100 M_2 plants since this method has been found to be the best for estimating mutation rates (9, 10).

**M_2 generation**

During the first rains of 1977, the suspected positive macro-mutants were carried to M_3. Each mutant was planted in a single row between two rows of the parental variety Clark-63. The spacing was 60 cm between rows and 10 cm within rows.

At harvest the mutant lines which were taller than the controls were harvested. The best plants were selected within each line and bulked.

The confirmed mutants were carried to M_4 for evaluation. On 18th October 1977, during the second rains, the confirmed mutants, Clark-63, a Clark isolate with a gene for delayed flowering, Congo-72, were planted in a complete randomised block experiment with four replicates. In each replicate, each mutant line occurred as a single row of nine metres long. The spacing was 60 x 60 cm.

Flowering date was recorded when approximately 50% of the plants in a line had their first flower and maturity date when approximately 95% of the pods were ripe.

At harvest, 10 plants from each row were used for determining plant height, number of branches, main-stem node number, and seed yield.

**RESULTS**

The mutagenic effect of low, medium and high doses of gamma-rays and EMS are presented in Table 1. The 5 krad of gamma-rays and 1.1% EMS caused about 10% growth reduction, while the 10 krad
of gamma-rays and 2.0% EMS caused approximately 50% growth reduction; the 45 krad of gamma-rays 96% growth reduction. The total number of seedlings that emerged in the M2 was significantly reduced in the 45 krad dose of gamma-rays and in both EMS treatments (P<0.05). Chlorophyll mutation increased with increase of gamma-ray and EMS dose. Positive macro-mutations were induced by all doses of gamma-rays and EMS although the 5 krad and 45 krad treatments did not differ statistically from the control (P>0.05). Medium doses of both mutagens induced higher frequencies of positive macro-mutations than their respective low doses (P<0.05).

At approximately 10% and 50% growth reduction levels, EMS was more efficient than gamma-rays for the induction of chlorophyll mutations and positive macro-mutations by a factor of 1.9 to 3.0 and 2.5 to 6.8 for chlorophyll and positive macro-mutations respectively.

The most promising mutants as revealed by the M4 evaluation are presented in Table 2 together with Clark-63, Congo-72 and a Clark isolate with a gene for delayed flowering. From the table, it can be seen that mutants with significantly increased vegetative period, days to maturity, plant height, number of branches and seed yield, in comparison with the parental variety, were induced by both gamma-rays and EMS (P<0.05).

Mutant lines EMS/2, 10 Kr/1 and 10 Kr/3 have yields comparable to the standard variety Congo-72, while mutant line 45 Kr/1 has seed yield significantly higher than Congo-72 (P<0.05). There was only a tendency for the gene for delayed flowering to increase seed yield.

By using plant height as the selection criterion it was possible to isolate mutants with increased vegetative period, days to maturity, main-stem node number, number of branches and seed yield.

The highest yielding mutant was induced by a high dose of gamma-rays (45 krad).

DISCUSSION

The observation made in Clark-63 that EMS is more efficient than gamma-rays for the induction of chlorophyll mutations, corresponds with the observations made by other investigators in other crop species; EMS induced mutations at rates higher than did sparsely ionising radiations (2,3,6,8,22,26,35,36). The results of the present investigation show that EMS is not only more efficient than gamma-rays for the induction of chlorophyll mutations, but also for the induction of positive macro-mutations.

The main objective of mutation breeders is to obtain maximum frequencies of positive mutations, but up to now there is no agreement on the most effective dose to use for the induction of maximum genetic variability, although most mutation breeders have preferred
Table 1: Chlorophyll mutation rates and positive macro-mutation rates induced by gamma-rays and EMS in the soybean variety Clark-63.

<table>
<thead>
<tr>
<th>Mutagen</th>
<th>Dose</th>
<th>$M_1$ growth reduction % of control</th>
<th>Total number of seedling in $M_2$</th>
<th>Chlorophyll mutation rate (%)</th>
<th>R.E. for chlorophyll mutations</th>
<th>Positive macro-mutation rate (%)</th>
<th>R.E. for positive macro-mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>-</td>
<td>6357 c</td>
<td>0 a</td>
<td>-</td>
<td>0 a</td>
<td>-</td>
</tr>
<tr>
<td>Gamma-rays</td>
<td>5 Krad</td>
<td>10.2</td>
<td>6375 c</td>
<td>0.24 b</td>
<td>-</td>
<td>0.05 a</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>10 Krad</td>
<td>47.6</td>
<td>5727 c</td>
<td>0.31 b</td>
<td>-</td>
<td>0.21 b</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>45 Krad</td>
<td>95.8</td>
<td>3720 a</td>
<td>0.89 d</td>
<td>-</td>
<td>0.08 a</td>
<td>-</td>
</tr>
<tr>
<td>EMS</td>
<td>1.1%</td>
<td>8.2</td>
<td>4698 b</td>
<td>0.58 c</td>
<td>1.9</td>
<td>0.26 b</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td>2.0%</td>
<td>49.9</td>
<td>3875ab</td>
<td>0.78cd</td>
<td>3.0</td>
<td>0.35 c</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Values followed by the same letter within the same column are not significantly different at $P = 0.05$ using Fishers LSD.

$$R.E. = \frac{M/G}{I} \frac{EMS}{gamma-rays}$$

where $M =$ mutation rate (%), $I =$ $M_1$ growth reduction (% of control)
to use medium doses \( (4,7,24,29,30,32,33,36) \) and the IAEA in their Manual on Mutation Breeding recommend medium doses \( (19,23) \). The fact that a medium dose \( (10 \text{ krad}) \) induced higher mutation rates of positive macro-mutants than did the low \( (10 \text{ krad}) \) or high \( (45 \text{ krad}) \) doses of gamma-rays in the present experiment, supports the popular view that medium doses should be used in general mutation induction experiments.

The fact that the highest yielding mutant was induced by a high dose of gamma-rays, suggests the use of high doses should not be ignored. However more practical evidence is still required.

<table>
<thead>
<tr>
<th>Mutants:</th>
<th>Days to flowering</th>
<th>Days to maturity</th>
<th>Plant height (cm)</th>
<th>Main-stem node number</th>
<th>Number of branches</th>
<th>Seed yield per plant (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMS/2</td>
<td>43</td>
<td>113</td>
<td>61.5</td>
<td>17.5</td>
<td>6.0</td>
<td>52.5</td>
</tr>
<tr>
<td>EMS/4</td>
<td>41</td>
<td>111</td>
<td>55.5</td>
<td>17.5</td>
<td>3.2</td>
<td>40.2</td>
</tr>
<tr>
<td>EMS/2/7</td>
<td>40</td>
<td>110</td>
<td>51.2</td>
<td>17.0</td>
<td>4.7</td>
<td>44.0</td>
</tr>
<tr>
<td>10 Kr/1</td>
<td>38</td>
<td>102</td>
<td>53.7</td>
<td>16.2</td>
<td>5.3</td>
<td>53.7</td>
</tr>
<tr>
<td>10 Kr/3</td>
<td>42</td>
<td>107</td>
<td>59.0</td>
<td>17.0</td>
<td>5.0</td>
<td>46.7</td>
</tr>
<tr>
<td>45 Kr/1</td>
<td>44</td>
<td>115</td>
<td>69.5</td>
<td>19.5</td>
<td>6.8</td>
<td>71.2</td>
</tr>
<tr>
<td>LSD at 5%</td>
<td>2.0</td>
<td>3.1</td>
<td>4.5</td>
<td>1.2</td>
<td>1.0</td>
<td>7.3</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

The EMS was donated by Prof. H. Gaul, Director of the Plant Breeding Institute at Grünbach, West Germany.

The seeds were irradiated by the IAEA, Vienna, Austria by Dr. H. Brunner.

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REFERENCES


Effects of Irradiation on Certain Characteristics of Shallots (Allium ascalonicum)

R. B. Dadson
Crop Science Dept., University of Ghana, Legon - Accra, Ghana.

ABSTRACT
Although shallots (A. ascalonicum) has been grown for a long time and used as a seasoning in many West African diets very little effort has been made to improve on yield and bulb size. The bulbs are so small that handling during food preparation is tedious and time consuming. It would, therefore, be desirable to improve the bulb size to that found in common onions.

The variability in bulb size of shallots has not been found sufficient to warrant a programme for selection. It has therefore, been necessary to explore the possibility of inducing mutations with irradiation, with the hope of recovering mutants which show increase in bulb size.

Irradiation studies using gamma rays of 10 to 500 rads indicated that shallots are very sensitive to irradiation. Beyond 500 rads the bulbs of M1 plants failed to sprout. There were increases in seedling death when dosage of gamma rays was increased. The number of bulbs per cluster, mean weight per cluster, weight per bulb, plant height and number of leaves per plant were all reduced by increase in dosage rate.

It is concluded that irradiation of shallots produced mainly deleterious effects.

INTRODUCTION

Shallot is a very important seasoning in the diet of many of the West African countries. The plant is small stunted and produces a cluster of bulbs from a single planted bulb. The bulbs are more pungent but
smaller than the common onion. Its handling, during food preparation is a tiresome job. The difficulty could be overcome if bulbs of bigger size could be found. Unfortunately, most of the cultivars have not shown any useful variability in bulb size which will make it worthwhile for selection to be undertaken.

Although the importance of inducing mutations through irradiation has been well established in many crops hardly has any use been made of this technique in creating variability in shallots. Irradiation of onions has been used only as a technique to prolong the shelf life as reported by Umeda: Takano and Sato (1970); Kalman (1975) and Kalman, Kiss and Farkas (1977). The most effective dosages were found to be from 5,000 rads and above. Using 5,000 rads as the highest dosage a mutation breeding programme in shallots was initiated in 1977 at Legon, to create variability which might include increase in bulb size.

Materials and Methods

Local shallots produced in the Keta region were obtained from Accra market and graded according to size 2 - 3 gm bulbs, 4 - 5 gm, and above 6 - 7 gm. The medium size bulbs 4 - 5 gm were selected and stored at 0°C for four weeks to break dormancy. They were irradiated at various dose-rates of gamma rays from a 60 Co source installed at the Ghana Atomic Energy Commission, Kwabenya. Eleven batches of 16 bulbs per batch were irradiated at 10, 50, 100; 200, 300, 400, 500, 750, 1,000, 2,500, and 5,000 rads. Sixteen unirradiated bulbs together with the irradiated bulbs were sown at a spacing of 9 ins x 9 ins on beds a few hours after irradiation. The nursery beds were irrigated immediately after planting and whenever necessary.

Germination records were taken from about five days until 21 days after planting. Other records taken
were number of bulbs reaching maturity, the number of bulbs per cluster, mean weight per cluster, mean plant height, and mean number of leaves.

The M1 plants were replanted and similar observations made on M2 plants and bulbs.

Results and Discussion

The results show that germination rate of M1 plants was not significantly affected between 0 and 400 rads treatments which were observed to give uniform germination. 500 rads and 1,000 to 5,000 rads completely inhibited germination, (Figure 1, 2, and 3). This agrees with what has been reported by Umeda; Takano and Sato (1970), Kalman (1975), Kiss and Tarkas (1977) who found 5,000 rads and above prolonged the storage life of onions.

It seems from this study that shallots are more sensitive to irradiation since germination seemed to be completely inhibited by exposure to dosages as little as 500 rads.

It was observed that a week after sprouting the leaves of some of the irradiated sets were yellowing. The sets which had yellowing leaves died after about ten days. The per cent survival to maturity decreased as the dosage of treatment was increased, (Table 1). The number of bulbs per cluster showed little variation as an effect of irradiation. 750 rads, however, greatly reduced the number of bulbs per cluster. The mean weight per cluster, weight per bulb, and plant height, were also drastically reduced by increasing the dosage (Table 2). The mean number of leaves per cluster was also reduced by increasing the rate of exposure. Irradiation appears to affect these plant characters adversely.

The drastic reduction in number of bulbs per cluster as the dosage rate was increased seems to arise from the effect of high dosages in preventing sprouting.
It is expected that a simultaneous reduction in plant height and number of leaves per plant would reduce the size of photosynthetic apparatus. This will cause a corresponding reduction in weight per cluster and weight per bulb as found in this study (Table 2).

In the M2 generation sets which had been obtained from bulbs exposed to 300 rads and above showed very weak sprouting, yellowing and died soon after sprouting. This is attributable to a latent effect of the irradiation treatment. There was a slight reduction in the number of bulbs per cluster, but there was an appreciable decrease in weight per cluster, weight per bulb, and number of leaves per cluster as dosage rate increased from 0 to 200 rads. This shows that the effect of irradiation treatment on these characters become established soon after treatment.

These findings indicate that irradiation of shallot bulbs would produce deleterious mutations as far as increasing bulb size and weight are concerned. These effects are caused through a reduction in number of sets in a cluster as well as the photosynthetic apparatus.

REFERENCES


**TABLE 1**

Effect of Irradiation on Growth of Shallots
*A. esculentum*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Germination</th>
<th>% Cluster Matured</th>
<th>No. of Bulbs per Cluster</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 rads</td>
<td>87.5</td>
<td>87.5</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>93.8</td>
<td>62.5</td>
<td>4</td>
</tr>
<tr>
<td>50</td>
<td>93.8</td>
<td>81.25</td>
<td>4</td>
</tr>
<tr>
<td>100</td>
<td>87.5</td>
<td>37.50</td>
<td>5</td>
</tr>
<tr>
<td>200</td>
<td>87.5</td>
<td>68.75</td>
<td>3</td>
</tr>
<tr>
<td>300</td>
<td>93.8</td>
<td>62.50</td>
<td>4</td>
</tr>
<tr>
<td>400</td>
<td>87.5</td>
<td>18.75</td>
<td>3</td>
</tr>
<tr>
<td>500</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
</tr>
<tr>
<td>750</td>
<td>37.5</td>
<td>31.25</td>
<td>1.5</td>
</tr>
<tr>
<td>1000</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
</tr>
<tr>
<td>2500</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
</tr>
<tr>
<td>5000</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
</tr>
</tbody>
</table>
### Table 2
Effect of Irradiation on Growth of Shallots
*A. ascalonicum*
M 1 Plants

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Weight Per Cluster (gm)</th>
<th>Mean Bulb Weight (gm)</th>
<th>Plant Height (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 rads</td>
<td>22.4</td>
<td>7.46</td>
<td>29.3</td>
</tr>
<tr>
<td>10</td>
<td>18.6</td>
<td>4.65</td>
<td>23.9</td>
</tr>
<tr>
<td>50</td>
<td>16.1</td>
<td>4.02</td>
<td>24.3</td>
</tr>
<tr>
<td>100</td>
<td>14.5</td>
<td>2.90</td>
<td>17.7</td>
</tr>
<tr>
<td>200</td>
<td>5.6</td>
<td>1.86</td>
<td>15.4</td>
</tr>
<tr>
<td>300</td>
<td>3.7</td>
<td>0.92</td>
<td>15.0</td>
</tr>
<tr>
<td>400</td>
<td>2.5</td>
<td>0.83</td>
<td>13.5</td>
</tr>
<tr>
<td>500</td>
<td>0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>750</td>
<td>1.5</td>
<td>0.39</td>
<td>10.5</td>
</tr>
<tr>
<td>1,000</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,500</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5,000</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 3
Effect of Irradiation on Growth of Shallots
*A. ascalonicum*
M 2 Plants

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bulbs/Cluster</th>
<th>Weight/Cluster (gm)</th>
<th>Weight/Bulb (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 rads</td>
<td>5.3</td>
<td>22.99</td>
<td>4.33</td>
</tr>
<tr>
<td>10</td>
<td>5.83</td>
<td>21.13</td>
<td>3.62</td>
</tr>
<tr>
<td>50</td>
<td>5.72</td>
<td>19.74</td>
<td>3.45</td>
</tr>
<tr>
<td>100</td>
<td>4.08</td>
<td>12.88</td>
<td>3.16</td>
</tr>
<tr>
<td>200</td>
<td>3.92</td>
<td>11.68</td>
<td>2.98</td>
</tr>
<tr>
<td>300 to 750</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Potential Use of Induced Mutations for Rice Improvement in Tanzania

Faculty of Agriculture, Forestry and Veterinary Science
University of Dar es Salaam, P.O. Box 643, Morogoro, Tanzania

Abstract
During the 1977 rainy season, $M_6$ mutants were selected on the basis of high yield and high grain protein potential. A further yield evaluation trial was then conducted at five locations in the 1978 season. Twenty two mutants and three control varieties were planted in February 1978. At least 10 mutants were found to be high yielding and high in seed protein content. A number of mutants were observed to be superior in protein content and grain yield to the parents. Correlation between yield, protein content and yield attributes were found to be non-significant, while that for protein % and protein per grain was highly significant. It has been possible in this study to identify correlation breakers which combine high grain yield potential and high protein content. The analysis of variance for yield, yield components and protein content and quality showed that there was a lot of similarity between mutant lines. Mutation breeding failed to reduce plant height in this study.

Introduction
Varietal selection and testing in rice has been done since the early thirties in Tanzania. Up to 1971 the aims of the rice breeding programme were to evaluate and recommend existing Tanzanian varieties; to isolate high yielding strains from one local variety and to select parents for a conventional hybridization programme.

The programme was expanded in 1972 to include breeding for higher protein content and quality in rice grain through induced mutations.

The need to have a big range of genotypes to use in the breeding programme together with the desire to increase the content and quality of plant protein in the human diet, prompted the utilization of induced mutation breeding. The aims of this programme were to improve the protein content in two high yielding local adapted rice varieties, namely Kihogo red and Faya Theresa; to incorporate lodging resistance and non-shattering in the variety Kihogo red and to select for fertilizer responsive, day neutral mutants from both cultivars. These varieties were treated with both chemical and physical mutagens at three doses each (Monyo, 1973). Results of $M_7$ generation will be discussed in this paper.
In 1978 further yield evaluation trials were done at Morogoro and at four other sites. The design and plant spacing were the same as that used in $M_6$ generation. Plot size was $3.6 \text{ m} \times 2.6 \text{ m}$. Fertilizers were applied at a rate of 120 kg N/ha and 50 kg P/ha. Nitrogen was applied in three equal split doses at planting, maximum tillering and booting stage while Phosphorus was applied in one dose at planting time. Data taken from the Morogoro site will be discussed.

Material and Methods

Two local cultivars, Paya Theresa and Kihogo red were sent to Vienna, in June 1972 for irradiation with gamma rays and fast neutrons. Three doses each of gamma rays and fast neutrons viz. 15 KRAD, 22.5 KRAD and 30 KRAD for gamma rays and 1.2 KRAD and 2.4 KRAD for fast neutrons respectively were used. Approximately 1,000 seeds were used for each dose rate. An equivalent amount of seed was used as control. The seeds were directly planted in well-prepared seed beds.

As for chemical mutagen, the seeds of the two varieties were soaked for twenty four hours at 20°C and then treated with 0.25%, 0.50% and 0.75% by volume of EMS in a constant water bath maintained at 30°C for six hours.

In both the irradiated and chemical treated seed material, the spacing was $15 \text{ cm} \times 15 \text{ cm}$ on the square and two to three seeds were planted per hole and later thinned to one. Plot size was $3 \text{ m} \times 4 \text{ m}$. Planting was done on 28 - 30 August, 1972, and harvesting done in April, 1973.

In early generations, i.e. $M_2$ and $M_3$ selection of mutants was based on agronomic characters i.e. flowering time, disease resistance, tiller number, plant height and plant vigour.

Screening for protein using DBC and micro-kjeldahl methods was initiated in $M_3$ generation (1974). Correlation between these two methods was found to be high (Monyo and Sugiyama 1975).

From $M_4$ and subsequent generations, selection was based on high yielding high protein mutants. In $M_5$ (1976) only 25 mutants were selected to constitute $M_6$ generation (1977). A yield evaluation trial was made at Morogoro using $M_6$ seed material in a triple lattice square design. The plot size was $3.6 \text{ m} \times 3.5 \text{ m}$ with a spacing of $20 \text{ cm} \times 20 \text{ cm}$ on the square.

Results

Data on grain yield components protein content and quality are
shown in Table I and II. The highest yielding mutant gave 7,863 kg/ha. Only three mutants gave yields below 6,000 kg/ha. Most of the mutants originating from Faya Theresa out yielded the parent variety. Only three Kihogo red mutants were higher yielding than the original variety.

Protein percent ranged from 8.9% to 10.3%. Grain protein percent in the untreated Faya Theresa and Kihogo red cultivars was 8.9% to 9.6% respectively. Lysine content was increased relative to the parent in some of the Faya Theresa mutants. In Kihogo red induced mutations appeared to decrease the lysine content.

The data showed that days to 50% emergence, tiller number per plant and plant height in mutants were similar to the parent varieties.

There was a positive and highly significant correlation between grain protein percent and protein per grain. Grain yield and grain protein showed a positive but non-significant correlation. Grain yield and number of effective tillers per plant were also highly correlated Table 3.

Discussion

The M₇ grain yield data was much higher than in previous generations. This was probably associated with the higher dose rate of nitrogen fertilizer applied to the 1978 season yield evaluation trial. As in previous generations the mutants maintained their higher ranking order relative to the mother varieties with regard to protein content (Monyo and Sugiyama, 1978). The higher grain yield in the mutants appeared to be related to the number of effective tillers per plant because there was a highly significant positive correlation between grain yield and effective tiller number per plant.

The results once again indicate that protein content in rice could be improved without sacrificing grain yield. The best yielding mutants were 22% higher in Faya Theresa and 27% higher in Kihogo red than in their respective mother varieties. In earlier generations the mutants were shown to be highly significant from the check varieties in yield. However the very high coefficient of variation observed in the M₇ data at Morogoro has made it difficult to make firm conclusions until the data from the other four locations has been analysed. This will also help to determine the extent of genotype-environment interaction.
References

Ellison, F.W. Pederson, D.C. and Devera, N.F. Selection for higher grain protein per cent in wheat. Proc. 3rd SABRAO Congress Canberra (1977) 2.5a-3b.


Monyo, J.H. Selection for higher grain yield and protein content in cereals. Proc. 3rd SABRAO Congress Canberra, 1977 5a-1.


Munk, L. Improvement of Nutritional Value in Cereals Heredities 72 (1972).


Table 8: Correlation Coefficients for different Components in Faya Theresa & 22.5 KRAD derived Mutants.

<table>
<thead>
<tr>
<th>Correlation Coefficients</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain Protein % and Grain Yield</td>
<td>0.19 n.s.</td>
</tr>
<tr>
<td>Grain Protein % and Protein per Grain</td>
<td>0.69**</td>
</tr>
<tr>
<td>Grain Protein % and 1000 grain weight</td>
<td>0.22 n.s.</td>
</tr>
<tr>
<td>Grain Yield and Protein per grain</td>
<td>0.21 n.s.</td>
</tr>
<tr>
<td>Grain Yield and 1000 grain weight</td>
<td>0.38 n.s.</td>
</tr>
<tr>
<td>Grain Yield and lysine</td>
<td>-0.03 n.s.</td>
</tr>
<tr>
<td>Grain Protein % and lysine</td>
<td>0.36 n.s.</td>
</tr>
<tr>
<td>% Protein and Plant Height</td>
<td>-0.09 n.s.</td>
</tr>
<tr>
<td>Grain Yield and effective tiller number per plant</td>
<td>0.77**</td>
</tr>
<tr>
<td>Protein content/tiller number per plant</td>
<td>0.31 n.s.</td>
</tr>
<tr>
<td>Grain Yield and Plant Height</td>
<td>+0.09 n.s.</td>
</tr>
</tbody>
</table>

n.s.;** = Non-significant and highly significant (p=0.01) respectively.
### Table 1: Grain Yield, Protein per cent, Protein per grain, Lysine content and Yield components.

<table>
<thead>
<tr>
<th>Entry No.</th>
<th>Variety</th>
<th>Mutagens/Dose</th>
<th>Yield kg/ha</th>
<th>Grain Protein %</th>
<th>Protein per grain</th>
<th>Lysine g/100g Protein</th>
<th>1000 grain weight (g)</th>
<th>Days to 50% Ear Emergencies</th>
<th>Tillers per plant</th>
<th>Average Plant Height (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>Faya Theresa &amp; 22.5 KRAD</td>
<td>-do-</td>
<td>7863</td>
<td>9.58</td>
<td>2.54</td>
<td>4.3</td>
<td>26.49</td>
<td>86</td>
<td>13</td>
<td>121</td>
</tr>
<tr>
<td>40</td>
<td>-do-</td>
<td>-do-</td>
<td>7831</td>
<td>9.49</td>
<td>2.46</td>
<td>3.6</td>
<td>25.96</td>
<td>94</td>
<td>14</td>
<td>137</td>
</tr>
<tr>
<td>26</td>
<td>-do-</td>
<td>-do-</td>
<td>7788</td>
<td>9.30</td>
<td>2.46</td>
<td>4.4</td>
<td>26.40</td>
<td>97</td>
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<td>122</td>
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<tr>
<td>35</td>
<td>-do-</td>
<td>-do-</td>
<td>7425</td>
<td>9.04</td>
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<td>102</td>
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<tr>
<td>6</td>
<td>-do-</td>
<td>-do-</td>
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<td>3.6</td>
<td>25.87</td>
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<tr>
<td>50</td>
<td>-do-</td>
<td>-do-</td>
<td>7308</td>
<td>9.36</td>
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<td>4.1</td>
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<td>7</td>
<td>-do-</td>
<td>-do-</td>
<td>7286</td>
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<td>26.69</td>
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<td>5</td>
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<td>-do-</td>
<td>6770</td>
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<td>96</td>
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<td>6581</td>
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<td>-</td>
<td>-do- &amp; Control</td>
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<td>17</td>
<td>-do-</td>
<td>E.M.S. 0.5%</td>
<td>5566</td>
<td>10.12</td>
<td>2.63</td>
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<td>25.75</td>
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<td>14</td>
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<td>Kihogo red Control</td>
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Mean: 6746

S.E. of the mean: + 328.35

Tukey Test 5%: + 1835

C.V.: - 5.3% - 12.3% - 5.6%
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<th>% Protein</th>
<th>Lysine</th>
<th>Flowering (50% E.M.)</th>
<th>Tillers per Plant</th>
<th>Height (cm)</th>
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S.E. of Mean ± 328 ± 3.0 ± 0.95 ± 3.8
Tukey Test (5%) ± 1835 ± 17 ± 5 ± 21.2
C.V. 25% 5% 12% 5.6%
<table>
<thead>
<tr>
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<th>Kg/ha</th>
<th>% Protein</th>
<th>Lysine</th>
<th>50% E.M.</th>
<th>Tillers per Plant</th>
<th>Plant Height (cm)</th>
</tr>
</thead>
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<tr>
<td>13 (&amp; 22.5 KRAD)</td>
<td>6955</td>
<td>9.1</td>
<td>3.7</td>
<td>98</td>
<td>14</td>
<td>122</td>
</tr>
<tr>
<td>36 (EMS 0.5%)</td>
<td>6367</td>
<td>10.3</td>
<td>3.6</td>
<td>100</td>
<td>14</td>
<td>118</td>
</tr>
<tr>
<td>31 (-do-)</td>
<td>6218</td>
<td>9.2</td>
<td>3.7</td>
<td>98</td>
<td>13</td>
<td>114</td>
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<tr>
<td>64 (Kihogo Red Control)</td>
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<td>4.7</td>
<td>100</td>
<td>12</td>
<td>122</td>
</tr>
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<td>S.E. of Mean</td>
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<td></td>
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<td>± 3</td>
<td>± 0.95</td>
<td>± 38</td>
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<td>Tukey Test (5%)</td>
<td>± 1835</td>
<td></td>
<td></td>
<td>± 17</td>
<td>± 5</td>
<td>± 21</td>
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<tr>
<td>C.V.</td>
<td>25%</td>
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<td>5%</td>
<td>12%</td>
<td>6%</td>
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Table 3: Correlations between Various Parameters in & 22.5 KRAD Faya Theresa Derived Mutants.

<table>
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<tr>
<th>Parameter 1</th>
<th>Parameter 2</th>
<th>Correlation Coefficient</th>
<th>Significance</th>
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<tr>
<td>% Grain protein with Grain Yield</td>
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<td>0.19</td>
<td>n.s.</td>
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<tr>
<td>-do- Protein per Grain</td>
<td></td>
<td>0.69**</td>
<td></td>
</tr>
<tr>
<td>Grain yield with Protein per Grain</td>
<td></td>
<td>0.21</td>
<td>n.s.</td>
</tr>
<tr>
<td>1000 grain weight with % Grain Protein</td>
<td></td>
<td>0.22</td>
<td>n.s.</td>
</tr>
<tr>
<td>-do- with Grain Yield</td>
<td></td>
<td>0.38</td>
<td>n.s.</td>
</tr>
<tr>
<td>Lysine Content with Grain Yield</td>
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<td>-0.09</td>
<td>n.s.</td>
</tr>
<tr>
<td>-do- % Protein Content</td>
<td></td>
<td>0.36</td>
<td>n.s.</td>
</tr>
<tr>
<td>% Grain Protein with Plant Height</td>
<td></td>
<td>-0.09</td>
<td>n.s.</td>
</tr>
<tr>
<td>Grain Yield with effective Tillers per Plant</td>
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<td>0.71**</td>
<td></td>
</tr>
<tr>
<td>% Protein Content with Tiller Number per Plant</td>
<td></td>
<td>0.31</td>
<td>n.s.</td>
</tr>
<tr>
<td>Grain Yield with Plant Height</td>
<td></td>
<td>0.09</td>
<td>n.s.</td>
</tr>
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</table>
RADIOSENSITIVITY OF SOME GOSYPium SPECIES
H. KHALIFA
COTTON BREEDING SECTION,
GEZIRA RESEARCH STATION,
WAD MEDANI
SUDAN

Abstract

RADIOSENSITIVITY OF SOME GOSYPium SPECIES.

Preliminary dose response experiments were carried out on dry seeds of some Gossypium species using 60Co gamma rays, fast and thermal neutrons to determine their radiosensitivity. Data were collected for characters related to physiological damage from the M1 populations. It was found that the selected species had different radiosensitivity to the same irradiation dose and to different types of irradiation. The diploid species were more sensitive than the amphidiploids. Fast and thermal neutrons were more destructive than 60Co gamma rays. Increasing dose of gamma irradiation revealed clear differences in physiological damage among the species. The relationships between the mean lengths of the hypocotyl and the first internode after irradiation, with increasing dose, were found to be linear.

1. INTRODUCTION

Cultivated cotton, beside being the most important fibre crop, is becoming a popular food crop because of its seed oil and high quality protein extracted from the seed cake. Improvement of a crop plant in productivity, quality, insect and disease resistance is possible through genetic variation available in the germplasm. If the desired genetic variation does not exist, then progress may be made through induced mutations.

For breeding purposes it is more desirable to have mutagenic treatments with low sterility effects but with high frequency of beneficial mutations. Therefore, the following preliminary experiments were carried out to check on the radiosensitivity of different Gossypium species and to determine the type and dose of irradiation which will yield low physiologic effects and strong genetic effects.
2. MATERIAL AND METHODS

Seeds of some amphidiploid cultivars and diploid wild species belonging to different genomes of *Gossypium* were collected from Shambat, Cotton Breeding Section, Herbarium, for irradiation. Dry seeds were used because they are easy to handle, and also to avoid any significant biological damage due to other pre- or post-environmental conditions.

2.1. Experiment I

Two hundred and fifty dry seed samples were taken from each of the cultivars and wild species shown in Table 1. The samples were irradiated at 11 krad of gamma rays (60Co source, Industrial Pilot Facility activity 29,000 Ci). Treatments were carried out, under constant conditions, at the Nuclear Energy Centre, Casaccia, Italy in 1974. The seeds were planted in the greenhouse at Shambat and later transplanted in the field when they were at the second leaf-stage. Data were collected on some characters related to physiological damage, and the radiosensitivity of the different genomes was determined. For each genome untreated sample was raised as a control.

2.2. Experiment II

Dry seeds of *G. anomalum* were used in this experiment. Six lots each of 250 dry seeds were irradiated with the above same 60Co gamma source at 5, 11, 14, 17 and 20 krad, with one untreated lot for each treatment as a control. Another 6 lots each of 250 dry seeds were treated with fast neutrons from (Tapiro Reactor, power 100 W, 800 Rad/h) at 0.5, 0.8, 1.1, 1.4, 1.7 and 2.0 krad, and untreated lots as controls for each treatment. Three other lots, 250 dry seeds each were irradiated with thermal neutrons derived from (Triga Mark II Reactor, 986 MeV, Flux $1.8576 \times 10^{13}$ cm$^2$/sec) at 3, 5 and 7 h. All treatments were carried out under constant environmental conditions at the Nuclear Energy Centre at Casaccia, Italy. Seeds were raised in the greenhouse, and the plants were transplanted in the field when they were at the second leaf-stage. Data were collected to determine the physiological damage due to the three types of radiation used.

2.3. Experiment III

For this experiment the New World amphidiploid cotton *G. hirsutum*, the Old World diploid cotton *G. herbaceum* and the
wild diploid cotton species *G. anomalum* were chosen. Irradiation using gamma rays from the $^{60}$Co source (Gamma Chamber 4000 A) at the Cotton Breeding Section, Shambat, at 0(control), 5, 10, 15, 20, 25, 30, 35, 40 and 45 krad, was carried out on dry seed samples (250 seeds each) collected from each of the three species. Treatments were carried out under constant environmental conditions. The seeds were raised in the greenhouse at Shambat and were transplanted in the field when they were at the second leaf-stage. Data were collected, at different developmental stages, for characters related to physiological damage. Curves showing the effect of increasing dose and the relationships among some of these characters were constructed.

3. RESULTS AND DISCUSSION

The diploid species of *Gossypium* ($2n=2x=26$) have been classified on the basis of cytological studies and geographical distribution into six genomes designated by the letters A to F and the amphidiploid species ($2n=4x=52$) by the letters (AD) which symbolized the two diploid original genomes (1,2).

The selected genomes in this study responded differently to the same dose (11 krad) of gamma irradiation. $M_1$ variant plants belonging to the diploid species were characterized by sectoring sterility suggesting that the apical meristem of a cotton plant consists of more than one cell and a mutation appeared in one cell and during differentiation gave rise to a male sterile branch. The remaining normal cells gave rise to fertile branches. This sterility is mainly due to chromosomal aberrations. Some plants of the amphidiploid species were characterized by wrinkled leathery leaves and excessive bud and flower shedding. Both the diploid and the amphidiploid species had plants with stunted growth. As shown in Table I the percentage of plants that died after germination was greater in the diploid than in the amphidiploid species, while the percentage $M_1$ variants was greater in the diploid species, especially in case of *G. herbaceum* ($A_1$-genome) and *G. thurberi* ($B_1$-genome). *G. stockii* ($B_1$-genome), being the most radiosensitive species, showed the least percentage of survivals. This is probably due to the fact that the amphidiploid species are more tolerant to chromosomal damage than the diploid species.

These results also indicate that the diploid species are more sensitive to gamma irradiation than the amphidiploid species. It has been reported (3) that nuclear volume, interphase chromosome volume and DNA content were the most
important biological factors governing radiosensitivity of plant species. Differences in radiosensitivity among genotypes within a species have also been reported (4), but it was not related to nuclear or interphase chromosome volume (5). Therefore, the results in this study suggest an inverse relationship between the ploidy level and radiosensitivity. The diploid species require less amount of energy to disrupt their genetic material as compared to the amphidiploid species.

Radiosensitivity of the same wild species (G.anomalum) was found to vary with different types of radiation. Table II shows that the percentages germination and plants that died after germination were found to be higher when the seeds were treated with either thermal or fast neutrons than with gamma rays. This is expected, as neutrons are known to be highly effective for the induction of mutation in plants. It was observed that plants treated with neutrons were characterized by retardation in growth rate, excessive bud and flower shedding, chlorophyll deficiency and complete sterility. This complete sterility indicates that all the apical meristem cells has mutated. The significance of this high sterility in the M₁ population is that it will limit the frequency of mutation in the succeeding generations (6). Therefore, gamma rays rather than neutrons is recommended for irradiation of cottonseed.

The effect of increasing the gamma rays dose on the physiological damage in G.hirsutum (cultivated amphidiploid), G.herbaceum (semi-wild diploid) and G.anomalum (wild diploid) revealed significant results. It can be seen in Table III that the per cent germination of G.hirsutum and G.herbaceum were not affected by increasing the radiation dose but G.anomalum seeds were relatively affected. The average length of hypocotyl of 10 days old seedlings of the three species decreased with the increase of the radiation dose. The average length of the first internode (20 days after planting) and the average length of the first leaf main vein (20 days after planting) decreased with the increasing dose, suggesting inverse relationships. In G.anomalum the seedlings that developed from seeds receiving 20 krad or more of gamma irradiation did not develop the first leaf, while in G.hirsutum the seeds receiving a radiation dose of 35 krad developed leaves. Generally the plants belonging to the three species showed distorted leaf lobing, leathery and varigated leaves and retarded apical meristem growth with increasing dose. Figure 1 depicts that G.anomalum has a higher rate of growth at the dose of 10 krad after which the average number of leaves per plant, 5 weeks after planting, decreases with the increase of dose, and at the radiation dose of 20 krad there are no survivals. But G.hirsutum and G.herbaceum show more or less uniform patterns of growth with the increase in radiation dose.
Figure 2 shows the effect of increasing radiation dose on the seedling mortality, 8 weeks after planting. Most of the plants belonging to *G. hirsutum* and *G. herbaceum* died at the cotyledonary stage, but in *G. anomalum* most deaths occurred before that stage. All the three species showed increased lethality with the increase in radiation dose. The 50% lethal dose (LD$_{50}$) for *G. hirsutum*, *G. herbaceum* and *G. anomalum* were 40, 30 and 7 krad, respectively. At 10 krad the seedling mortality for *G. anomalum* is about 80% and it reaches 100% at 20 krad.

The surviving plants, 12 weeks after planting, also decrease with the increase of radiation dose. At a dose of 10 krad almost 85% of the plants survived in case of *G. hirsutum*, while in *G. anomalum* the survivals are 10% (Fig. 3).

The correlations between the length of the hypocotyl and survivals ($r=0.86$, $p=0.01$), and between the length of the first internode and survivals ($r=0.74$, $p=0.01$) are positive and highly significant. The relationships between survival rate and lengths of hypocotyl and first internode after gamma ray treatments with increasing doses are found to be linear (Figs. 4, 5). It was reported that acute doses of gamma irradiation of maize pollen resulted in a non-linear relationship (7).

ACKNOWLEDGEMENT

I am most grateful to Professor B. Donini for carrying out seed treatment for the first two experiments at the Nuclear Research Centre, Cassacia, Italy.
REFERENCES

(1) BEASLEY, J.O. Meiotic chromosome behaviour in species hybrids, haploids, and induced polyploids of Gossypium, Genetics 27 (1942) 25.


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<thead>
<tr>
<th>Genome</th>
<th>No. of seeds treated</th>
<th>Germination (%)</th>
<th>Plants died after germination (%)</th>
<th>Survivals (%)</th>
<th>M₁ Variants (%)</th>
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<td>69</td>
<td>21</td>
<td>48</td>
<td>7</td>
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<td>250</td>
<td>74</td>
<td>22</td>
<td>44</td>
<td>6</td>
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<tr>
<td>G. hirsutum L. (Acala) (AD)²</td>
<td>250</td>
<td>98</td>
<td>14</td>
<td>58</td>
<td>11</td>
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<tr>
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<td>250</td>
<td>61</td>
<td>30</td>
<td>31</td>
<td>7</td>
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<tr>
<td>G. herbaceum L. A₁</td>
<td>250</td>
<td>71</td>
<td>66</td>
<td>43</td>
<td>26</td>
</tr>
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<td>250</td>
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<td>42</td>
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<tr>
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<td>37</td>
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<td>250</td>
<td>54</td>
<td>65</td>
<td>42</td>
<td>16</td>
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<td>G. thurberi Tod. D₁</td>
<td>250</td>
<td>41</td>
<td>58</td>
<td>27</td>
<td>29</td>
</tr>
<tr>
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<td>250</td>
<td>59</td>
<td>65</td>
<td>7</td>
<td>14</td>
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TABLE II. RADIOSENSITIVITY OF G. ANOMALUM TO GAMMA, FAST AND THERMAL NEUTRONS IRRADIATION

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Gamma rays $^a$ (krad)</th>
<th>Fast Neutrons $^a$ (krad)</th>
<th>Thermal neutrons $^a$ (h)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>5  8  11 14 17 20</td>
<td>0.5 0.8 1.1 1.4 1.7 2.0</td>
<td>3  5  7</td>
</tr>
<tr>
<td>Germination (%)</td>
<td>68 99 89 68 78 69 48 3 25 23 13 10 7 8 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plants died after germination (%)</td>
<td>38 47 54 13 34 50 63 83 54 70 72 92 93 97 70</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$Two hundred and fifty dry seed-samples.
<table>
<thead>
<tr>
<th>Radiation (krad)</th>
<th>% of seed germination</th>
<th>\text{C. hirsutum}(^a) Hypocotyl length(^b) (mm)</th>
<th>\text{C. hirsutum}(^a) Length of first internode(^b) (mm)</th>
<th>% of seed germination</th>
<th>\text{C. hirsutum}(^a) Hypocotyl length(^b) (mm)</th>
<th>\text{C. hirsutum}(^a) Length of first internode(^b) (mm)</th>
<th>% of seed germination</th>
<th>\text{C. hirsutum}(^a) Hypocotyl length(^b) (mm)</th>
<th>\text{C. hirsutum}(^a) Length of first internode(^b) (mm)</th>
<th>% of seed germination</th>
<th>\text{C. hirsutum}(^a) Hypocotyl length(^b) (mm)</th>
<th>\text{C. hirsutum}(^a) Length of first internode(^b) (mm)</th>
<th>% of seed germination</th>
<th>\text{C. hirsutum}(^a) Hypocotyl length(^b) (mm)</th>
<th>\text{C. hirsutum}(^a) Length of first internode(^b) (mm)</th>
<th>% of seed germination</th>
<th>\text{C. hirsutum}(^a) Hypocotyl length(^b) (mm)</th>
<th>\text{C. hirsutum}(^a) Length of first internode(^b) (mm)</th>
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</tr>
</tbody>
</table>

\(^a\) Ten days after planting
\(^b\) Twenty days after planting
\(^c\) Twenty days after planting
\(^d\) Two hundred and fifty- five dry seed-sample
Fig. 1. Effect of increasing dose of gamma irradiation on the number of leaves per plant of three species of Gossypium (5 weeks old).
Fig. 2. Effect of increasing dose of gamma irradiation on the seedling mortality of three species of *Gossypium* (8 weeks old).
Fig. 3. Dose effect of gamma irradiation on the surviving plants of three species of *Gossypium* (12 weeks old).
The relation between the surviving plants and mean length of hypocotyl for G. hirsutum treated with increasing dose of gamma irradiation.

Fig. 4.
$y = 1.82 + 1.447 \times$

Fig. 5. The relation between the surviving plants and mean length of first internode for *G. hirsutum* treated with increasing dose of gamma irradiation.
ETUDE DE COMPORTEMENT DE QUELQUES LIGNÉES MUTANTES DE SOJA DANS LE NORD DE L'ALGÉRIE

NICOLAE Ion
OUGOUAG Bachir

Institut National Agronomique d'Alger (El-Harrach)

Abstract

Soya has the potential of becoming a very important source of protein and oil for human nutrition, animal feed and for industrial purposes. Since 1974, the government is concerned with introduction and popularization of soybean cultivation. In 1975, work on soybean mutants started with planting mutant lines obtained from Roumanie in large scale trials. Results of these trials are reported.

INTRODUCTION


En Algérie les recherches sur l'amélioration du soja par mutation ont commencé récemment par la sélection de mutants favorables dans une collection de lignées issues de semences irradiées en Roumanie et expérimentées pendant quatre années à l'Institut National Agronomique d'Alger et à la Station Expérimentale de Khemis-Miliana (Haut-Cheliff).

MATERIEL ET METHODE DE TRAVAIL

1. Matériel biologique utilisé

Nous avons utilisé comme matériel biologique 15 lignées de soja retenues après deux ans d'expérimentation pour leur précocité et leurs rendements satisfaisants.


Dans nos essais nous avons utilisé comme témoin la lignée B 107/10 dont sont issues ces lignées expérimentées en Algérie.
2. Méthode de travail

Nos recherches concernant l’évolution de quelques lignées mutantes de soja ont été effectuées en 1976 à l’Institut National Agronomique d’Alger, sous climat de la Mitidja et sur un sol de type rouge méditerranéen.

Les lignées de soja ont été semées à l’époque optimale (3 avril) manuellement, grain par grain, dans un dispositif expérimental de type "blocs randomisés", en trois répétitions. La superficie "active" de la parcelle élémentaire a été de 8 m². L’expérimentation de ces lignées a été faite en régime sec (non irrigué).

Nous avons analysé les différentes lignées de soja en fonction de leurs caractères morphologiques, physiologiques et biochimiques, afin de pouvoir établir les meilleures formes adaptées aux conditions de la Mitidja (nord de l’Algérie).


En ce qui concerne les analyses biochimiques, nous avons déterminé le taux de protéines brutes et de matières grasses. Un lot moyen de graines de chaque mutant a été broyé jusqu’à l’obtention d’une mouture fine. Sur cette farine nous avons déterminé le taux d’azote total par le procédé Kjéldahl; la teneur en protéine brutes a été calculé en multipliant ce taux par le coefficient 6,25. Le dosage des matières grasses est effectué dans des vases de soxhlet où l’extraction à l’éther diéthylélique a duré 6 heures à une température de 55°C.

Après la récolte, nous avons déterminé le rendement de toutes les parcelles en Kg/ha et à l’aide de l’analyse de la variance, nous avons calculé les significations des différences entre chaque lignée et le témoin.

RÉSULTATS OBTENUS ET DISCUSSIONS

1. Analyse des principaux caractères morphologiques.
   a) Caractères de la plantule (tableau 1).
   À ce stade de végétation nous distinguons les différences pour certains mutants au niveau de la coloration et de la taille des différentes parties de la plantule.

   b) Caractères de la plante adulte (tableau 2).
   Parmi les caractères de la plante adulte nous avons examiné ceux qui diffèrent nettement par rapport au témoin (la variété initiale) et qui sont très souvent utilisés pour la description et la classification de nouvelles formes de soja. Ainsi nous avons fait des notations sur les principaux caractères de feuilles, les caractères de la fleur et les caractères de la plante (taille, pilosité, forme, port).

   Il est à noter que la plus part des lignées mutantes diffèrent par un ou plusieurs caractères par rapport au témoin.
### Tableau N° 1

**Caractères de la plantule et de la jeune plante.**

<table>
<thead>
<tr>
<th>Variétés</th>
<th>Coloration</th>
<th>Hypocotyle (Pilosité)</th>
<th>Feuilles Primaires (Taille)</th>
<th>Pétiole (Coloration)</th>
<th>Pétiolule (Coloration)</th>
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<td>Forte</td>
<td>Moyenne</td>
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<td>Forte</td>
<td>Moyenne</td>
<td>Violet</td>
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</tr>
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<td>Forte</td>
<td>Grande</td>
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<td>Non coloré</td>
</tr>
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<td>Forte</td>
<td>Moyenne</td>
<td>Non coloré</td>
<td>Non coloré</td>
</tr>
<tr>
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<td>Non coloré</td>
<td>Non coloré</td>
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<tr>
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<td>Forte</td>
<td>Grande</td>
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<tr>
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<td>Non coloré</td>
</tr>
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</tr>
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<td>Non coloré</td>
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<tr>
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<td></td>
<td>Coloration</td>
<td>Coloration</td>
<td>Forme</td>
<td>Angle Tige-pétiole</td>
<td>Forme de la plante</td>
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<tr>
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<td>Ovale</td>
<td>Moyenne</td>
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<tr>
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<td>Vert</td>
<td>Ovale</td>
<td>Petite</td>
</tr>
<tr>
<td>79/15</td>
<td>Fauve</td>
<td>Blanche</td>
<td>Vert</td>
<td>Ovale</td>
<td>Grande</td>
</tr>
<tr>
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<td>Blanche</td>
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</tr>
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</tr>
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<td>Blanche</td>
<td>Vert</td>
<td>Ovale</td>
<td>Putte</td>
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<tr>
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<td>Blanche</td>
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</tr>
<tr>
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<td>Blanche</td>
<td>Vert</td>
<td>Ovale</td>
<td>Moyenne</td>
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<tr>
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<td>Violacée</td>
<td>Vert</td>
<td>Ovale</td>
<td>Moyenne</td>
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</table>
o) Caractères des gousses (tableau 3).

La coloration, la forme et l'étranglement des gousses sont les caractères morphologiques que nous avons examinés sur notre collection. Ces caractères diffèrent d'une lignée à l'autre de façon plus ou moins évidentes.

d) Caractères du grain (tableau 4) sont très importants pour la description et la classification des nouvelles variétés, étant donné leur stabilité génétique.

- Coloration du tégument: Dans notre collection, la coloration ne présente pas de grandes variations d'une variété à l'autre. Nous distinguons 3 types de couleurs: jaune, jaune pâle et jaune à marron clair.

- Panachures: Suivant les variétés, nous remarquons qu'il existe des panachures de couleurs différentes ainsi que leur étendue autour du hile.

 Dans un lot de semences d'une même variété, des graines peuvent être ou ne pas être fortement panachées. Nous avons retenu deux types de panachures: marron et noir.
- Aspect du tégument: Il existe des variétés qui présentent un aspect mat, mais chez nos lignées les grains avaient un aspect lustré.
- Forme du grain: Nous avons retenu 2 formes: ovale et ovale à arrondie.
- Cavité lenticulaire: C'est une dépression qui s'observe chez un certain nombre de variétés après avoir décortiqué le grain, au centre de la face convexe des cotylédons. La cavité lenticulaire peut être nulle ou marquée.
- Funicule: Il est situé au niveau du hile, facile à observer.
We remarquons que sur certaines lignées le funicule est persistant alors que sur d'autre il est caduque.
- Hile: La coloration du hile est très stable suivant la variété. Sur les lignées que nous avons étudiées, le hile est noir, marron foncé, marron ou jaune.
- La ligne médiane divise le hile en 2 parties égales dans le sens longitudinal. Sa couleur variant du jaune au blanc.
- La taille du hile varie avec les variétés. Nous avons retenu 2 types de taille du hile par rapport à celle du grain: moyen et petit.

2. Analyse des principaux caractères physiologiques.

Pour la détermination des caractères physiologiques nous avons basé les résultats sur l'expérience. Les appréciations portant sur le pourcentage de plantes affectées pendant les stades critiques de végétation. En fonction de ce pourcentage nous avons accordé des qualificatifs (tableau 5 )

a) Résistance au froid printanier.

Pour examiner ce caractère très important surtout pour la zone de haut Chellif, nous avons effectué une époque de semis coïncidant avec une température du sol inférieure à 8°C. Nous avons constaté que généralement les variétés plus tardives sont résistantes, alors que la majorité des variétés précoces sont sensibles, ne pouvant pas germer et leurs grains pourrissent vite. Cependant nous avons constaté que deux lignées précoces sont très résistantes au froid printanier (B 62/15 et B 10/37), étant capable de germer facilement à une température de 6°-8°C et de supporter les variations de température après la levée des plantes.

b) Résistance à la sécheresse.

Pour l'analyse de ce caractère aussi limitant l'extension du soja en Algérie, tant en régime sec qu'en irrigué, nous avons observé les lignées mutantes pendant la période de floraison quand la sécheresse a été bien prolongé et surtout après le sirocco. Ainsi nous avons pu constater l'effet caractéristique de la sécheresse (fletrissement et jaunissement des feuilles) sur les lignées sensibles. Après la récolte nous avons examiné aussi le pourcentage des grains ridés, très fortement prononcé pour certaines lignées.

Dans tableau nr. 5 on constate que les lignées B 62/15 et B 10/37, sont très résistantes et c'est pourquoi actuellement elles sont expérimentées en régime sec dans le nord de l'Algérie.

Soil Résistance à la vorse.

Avant la récolte, nous avons pu constater que certaines lignées étaient plus résistante à la vorse que d'autres. Dans notre essais, la vorse a été dû à des facteurs naturels (vent, pluie ....).
# Tableau 4

## Caractères du grain

<table>
<thead>
<tr>
<th>Variétés</th>
<th>Caractères</th>
<th>Tégument (Coloration)</th>
<th>Pannache</th>
<th>Tégument</th>
<th>Forme</th>
<th>Cavité Lenticulaire</th>
<th>Punicule</th>
<th>Hile (Coloration)</th>
<th>Ligne médiane (Coloration)</th>
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<td>Jaune pêle</td>
<td>Marron</td>
<td>Lustré</td>
<td>Ovale</td>
<td>Nullo</td>
<td>Persistant</td>
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<td>Blanche</td>
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<td>Lustré</td>
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<td>Caduque</td>
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<td>Ovale</td>
<td>Marqué</td>
<td>Caduque</td>
<td>Marron</td>
<td>Jaune</td>
<td></td>
</tr>
<tr>
<td>10/37</td>
<td>Jaune à Jaune pêle</td>
<td>Marron</td>
<td>Lustré</td>
<td>Ovale</td>
<td>Marqué</td>
<td>Caduque</td>
<td>Marron</td>
<td>Jaune</td>
<td></td>
</tr>
<tr>
<td>10/8</td>
<td>Jaune à marron pêle claire</td>
<td>Marron</td>
<td>Lustré</td>
<td>Ovale</td>
<td>Nullo</td>
<td>Persistant</td>
<td>Marron</td>
<td>Jaune</td>
<td></td>
</tr>
<tr>
<td>90/40</td>
<td>Jaune</td>
<td>Marron</td>
<td>Lustré</td>
<td>Ovale</td>
<td>Nullo</td>
<td>Caduque</td>
<td>Jaune</td>
<td>Blanche</td>
<td></td>
</tr>
<tr>
<td>10/4</td>
<td>Jaune</td>
<td>Marron</td>
<td>Lustré</td>
<td>Ovale</td>
<td>Nullo</td>
<td>Caduque</td>
<td>Jaune</td>
<td>Blanche</td>
<td></td>
</tr>
<tr>
<td>62/15</td>
<td>Jaune</td>
<td>Marron</td>
<td>Lustré</td>
<td>Ovale</td>
<td>Nullo</td>
<td>Caduque</td>
<td>Marron</td>
<td>Jaune</td>
<td></td>
</tr>
<tr>
<td>71/71</td>
<td>Jaune</td>
<td>Marron</td>
<td>Lustré</td>
<td>Ovale</td>
<td>Nullo</td>
<td>Caduque</td>
<td>Marron</td>
<td>Noir</td>
<td>Blanche</td>
</tr>
<tr>
<td>79/35</td>
<td>Jaune pêle</td>
<td>Marron</td>
<td>Lustré</td>
<td>Ovale</td>
<td>Nullo</td>
<td>Caduque</td>
<td>Marron</td>
<td>Jaune</td>
<td></td>
</tr>
<tr>
<td>10/11</td>
<td>Jaune à marron pêle claire</td>
<td>Marron</td>
<td>Lustré</td>
<td>Ovale à arrondie</td>
<td>Nullo</td>
<td>Caduque</td>
<td>Marron</td>
<td>Jaune</td>
<td></td>
</tr>
</tbody>
</table>
ANALYSE DES CARACTÈRES PHYSIOLOGIQUES.

Tableau 5.

<table>
<thead>
<tr>
<th>CARACTÈRES PHYSIOLOGIQUES</th>
<th>Résistance à la sécheresse</th>
<th>Résistance à la verse</th>
<th>Résistance au gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variétés</td>
<td>Résistant</td>
<td>Résistant</td>
<td>Résistant</td>
</tr>
<tr>
<td>107/10 (m)</td>
<td>Très sensible</td>
<td>Résistant</td>
<td>Résistant</td>
</tr>
<tr>
<td>90/50</td>
<td>Sensible</td>
<td>Résistant</td>
<td>Résistant</td>
</tr>
<tr>
<td>90/40</td>
<td>Résistant</td>
<td>Résistant</td>
<td>Résistant</td>
</tr>
<tr>
<td>90/20</td>
<td>Résistant</td>
<td>Résistant</td>
<td>Sensible</td>
</tr>
<tr>
<td>90/10</td>
<td>Résistant</td>
<td>Très résistant</td>
<td>Résistant</td>
</tr>
<tr>
<td>79/15</td>
<td>Résistant</td>
<td>Sensible</td>
<td>Résistant</td>
</tr>
<tr>
<td>79/35</td>
<td>Résistant</td>
<td>Résistant</td>
<td>Très résistante</td>
</tr>
<tr>
<td>71/71</td>
<td>Très sensible</td>
<td>Sensible</td>
<td>Résistant</td>
</tr>
<tr>
<td>10/35</td>
<td>Très résistant</td>
<td>Résistant</td>
<td>Sensible</td>
</tr>
<tr>
<td>10/37</td>
<td>Très résistant</td>
<td>Très résistant</td>
<td>Résistant</td>
</tr>
<tr>
<td>10/4</td>
<td>Résistant</td>
<td>Résistant</td>
<td>Sensible</td>
</tr>
<tr>
<td>10/3</td>
<td>Très résistant</td>
<td>Résistant</td>
<td>Sensible</td>
</tr>
<tr>
<td>10/11</td>
<td>Résistant</td>
<td>Résistant</td>
<td>Sensible</td>
</tr>
<tr>
<td>10/55</td>
<td>Résistant</td>
<td>Résistant</td>
<td>Très résistante</td>
</tr>
<tr>
<td>62/15</td>
<td>Très résistant</td>
<td>Très résistant</td>
<td>Très résistante</td>
</tr>
</tbody>
</table>

d) L'époque de maturité.

Nous avons attendu la maturité complète pour faire la récolte. Bien que le semis a été fait à la même date pour toutes les lignées, la récolte a été échelonnée selon les variétés. En nombre de jours pour trois ans d'expérimentation nous avons les moyennes suivantes:

<table>
<thead>
<tr>
<th>Variétés</th>
<th>107</th>
<th>90</th>
<th>90</th>
<th>10</th>
<th>79</th>
<th>10</th>
<th>10</th>
<th>90</th>
<th>10</th>
<th>62</th>
<th>71</th>
<th>79</th>
<th>10</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>50</td>
<td>20</td>
<td>10</td>
<td>35</td>
<td>15</td>
<td>37</td>
<td>8</td>
<td>40</td>
<td>4</td>
<td>15</td>
<td>71</td>
<td>35</td>
<td>55</td>
</tr>
<tr>
<td>Nombre de jours</td>
<td>91</td>
<td>110</td>
<td>119</td>
<td>96</td>
<td>110</td>
<td>98</td>
<td>97</td>
<td>90</td>
<td>116</td>
<td>106</td>
<td>97</td>
<td>120</td>
<td>95</td>
<td>99</td>
</tr>
</tbody>
</table>

Dans ce tableau on constate que la majorité des variétés sont précoce, à l'exception de 71/71, 90/50, 90/20 et 10/11.

e) Les aptitudes à la récolte mécanique.

Si l'insertion de la première gousse a la base de la plante est très basse, on peut craindre qu'elles ne soient pas récoltées lors du passage de la moissonneuse-batteuse. Les variétés, dont l'insertion de la première gousse atteint au minimum 9 cm conviennent à cette récolte mécanique. Dans notre collection la hauteur moyenne de la première gousse varie de 6,10 cm à 12,90 cm (tableau 6).
Tableau 6

Hauteur d'insertion de la première gousse

<table>
<thead>
<tr>
<th>Variétés</th>
<th>T</th>
<th>90/50</th>
<th>90/20</th>
<th>90/10</th>
<th>10/35</th>
<th>79/15</th>
<th>10/37</th>
<th>10/8</th>
<th>90/40</th>
<th>10/4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hauteur de la 1ère gousse</td>
<td>79/35</td>
<td>10/5</td>
<td>10/11</td>
<td>62/15</td>
<td>71/71</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6,1</td>
<td>7,2</td>
<td>8,2</td>
<td>7,6</td>
<td>12,9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

On constate que trois variétés conviendraient bien à la récolte mécanique (10/35, 10/8 et 71/71), toutefois, en sol bien nivelé on pourrait leur ajouter les variétés 90/20, 79/15, 10/37, 90/50, 90/4 et 62/15.

3. Caractères biochimiques

Nous avons déterminé le taux de protéines et de matières grasses, pour permettre une caractérisation variétale plus précise.

a) Teneur en protéines des lignées étudiées varie de 35,06 à 45,81 (tableau 7). La variété témoin (107/10) présente un taux protéique très élevé par rapport aux autres variétés à l'exception de la 10/37 (taux protéique supérieur au témoin).

Compte tenu de ces résultats, nous prendrons comme géniteurs, pour la recherche de taux protéiques élevés, les 2 variétés (10/37 et 107/10).

b) Teneur en matière grasse.

Le pourcentage en lipide des différentes variétés étudiées varie de 14,50 à 23, 50% de matière sèche (tableau 8). Les lignées 107/10, 10/37, 10/35, 62/15 et 10/4 ont une teneur lipidique assez faible, bien que l'ensemble des variétés sont supérieure au témoin.

Les lignées présentent une teneur lipidique élevée (90/10, 90/50 et 10/11) pourront servir comme géniteur.

En général, les variétés qui ont une teneur en protéine élevée, ont une teneur lipidique faible.

4. Comportement des variétés (lignées) sur le rendement en grains.

L'indice le plus synthétique, et exact, concernant la valeur productive d'une variété est sans doute le rendement en grains par ha. Le tableau nr. 8 montre que du point de vue de ce facteur, les variétés les plus intéressantes sont B 62/15, B 90/10 et B 10/35. Ces variétés sont également très importantes pour leur précocité et leur résistance aux facteurs du milieu.

Au bout de trois ans d'expérimentation nous recommandons ces variétés pour la multiplication de semences et pour leur expérimentation dans d'autres régions pédo-climatiques. De plus, les variétés B 62/15 et B 10/35 ont donnés de très bons résultats à El - Khemis.
### Tableau 7

**Protéines (en % de M.S.)**

Des variétés de soja étudiées.

<table>
<thead>
<tr>
<th>Variétés</th>
<th>Azote en %</th>
<th>Taux de Protéine</th>
<th>Différence</th>
</tr>
</thead>
<tbody>
<tr>
<td>T (107/10)</td>
<td>7,16</td>
<td>44,75</td>
<td>0</td>
</tr>
<tr>
<td>90/50</td>
<td>5,61</td>
<td>35,06</td>
<td>- 9,69</td>
</tr>
<tr>
<td>90/20</td>
<td>6,30</td>
<td>39,37</td>
<td>- 5,38</td>
</tr>
<tr>
<td>90/10</td>
<td>6,08</td>
<td>38,00</td>
<td>- 6,75</td>
</tr>
<tr>
<td>10/35</td>
<td>6,71</td>
<td>41,93</td>
<td>- 2,82</td>
</tr>
<tr>
<td>79/15</td>
<td>6,85</td>
<td>42,81</td>
<td>- 1,94</td>
</tr>
<tr>
<td>10/37</td>
<td>7,33</td>
<td>45,81</td>
<td>+ 1,06</td>
</tr>
<tr>
<td>10/6</td>
<td>6,85</td>
<td>42,81</td>
<td>- 1,94</td>
</tr>
<tr>
<td>90/40</td>
<td>6,56</td>
<td>41,00</td>
<td>- 3,75</td>
</tr>
<tr>
<td>10,4</td>
<td>6,76</td>
<td>42,25</td>
<td>- 2,50</td>
</tr>
<tr>
<td>62/15</td>
<td>6,48</td>
<td>40,50</td>
<td>- 4,25</td>
</tr>
<tr>
<td>71/71</td>
<td>6,16</td>
<td>38,62</td>
<td>- 6,13</td>
</tr>
<tr>
<td>79/35</td>
<td>6,22</td>
<td>38,87</td>
<td>- 5,88</td>
</tr>
<tr>
<td>10/55</td>
<td>5,95</td>
<td>37,18</td>
<td>- 7,17</td>
</tr>
<tr>
<td>10/11</td>
<td>6,05</td>
<td>37,81</td>
<td>- 6,94</td>
</tr>
</tbody>
</table>

### Tableau 8

**Matières Grasses en % de M.S.**

Des variétés de soja étudiées.

<table>
<thead>
<tr>
<th>Variétés</th>
<th>Lipides en %</th>
<th>Différence</th>
</tr>
</thead>
<tbody>
<tr>
<td>T (107/10)</td>
<td>14,50</td>
<td>0</td>
</tr>
<tr>
<td>90/50</td>
<td>22,00</td>
<td>+ 7,50</td>
</tr>
<tr>
<td>90/20</td>
<td>20,50</td>
<td>+ 6,00</td>
</tr>
<tr>
<td>90/10</td>
<td>23,50</td>
<td>+ 9,00</td>
</tr>
<tr>
<td>10/35</td>
<td>17,25</td>
<td>+ 2,75</td>
</tr>
<tr>
<td>73/15</td>
<td>19,50</td>
<td>+ 5,00</td>
</tr>
<tr>
<td>10/37</td>
<td>17,25</td>
<td>+ 2,75</td>
</tr>
<tr>
<td>10/8</td>
<td>18,75</td>
<td>+ 4,25</td>
</tr>
<tr>
<td>90/40</td>
<td>18,50</td>
<td>+ 4,00</td>
</tr>
<tr>
<td>10/4</td>
<td>16,00</td>
<td>+ 1,50</td>
</tr>
<tr>
<td>62/15</td>
<td>16,75</td>
<td>+ 2,25</td>
</tr>
<tr>
<td>71/71</td>
<td>19,75</td>
<td>+ 5,25</td>
</tr>
<tr>
<td>79/35</td>
<td>19,50</td>
<td>+ 5,00</td>
</tr>
<tr>
<td>10/55</td>
<td>20,50</td>
<td>+ 6,00</td>
</tr>
<tr>
<td>10/11</td>
<td>21,25</td>
<td>+ 6,75</td>
</tr>
</tbody>
</table>
CONCLUSIONS ET RECOMMANDATIONS

L'étude variétale a fait l'objet de nombreuses recherches dans les pays développés, ainsi que dans certains pays africains à savoir Nigeria, Maroc, Tunisie, Congo, etc... En Algérie où les recherches avaient débuté par l'établissement d'une collection de variétés (Blanchard, 1956), la situation politique ne pouvait permettre aux chercheurs de poursuivre leurs travaux.

A cet effet, nous avons convenu de suivre en quelque sorte ce travail pouvant servir de base à la continuation sur l'étude variétale et la sélection des nouvelles formes de soja mieux adaptées en Algérie.

Les variétés de soja étudiées étant expérimentées dans un même milieu, l'analyse des caractères morphologiques nous a permis de les comparer et d'avoir beaucoup de renseignements sur chacune d'elles.

L'analyse des caractères physiologiques nous a permis de connaître la résistance des variétés (linées) étudiées aux facteurs du milieu, ainsi que la précocité - particularité la plus essentielle pour l'extension du soja en Algérie tant en régime irrigué qu'en sec.
L'analyse biométrique que nous avons effectué, a permis de mettre en évidence des différences sur les principales composantes du rendement, pour chaque variété. Les calculs de la variance et du coefficient de variabilité ont montré la plus ou moins grande homogénéité des caractères de chaque variétés et la possibilité de les sélectionner.

Dans l'étude des caractères biochimiques nous avons identifié les formes riches en protéines et en acides aminés. Cela nous permettra de sélectionner les formes intéressantes de ce point de vue ou de les utiliser comme géniteurs.

Le comportement des variétés du point de vue rendement en grains montre la supériorité des lignées B 62/15, B 90/10, B 10/55. C'est pourquoi nous pouvons d'ores et déjà recommander ces formes afin de les introduire dans l'agriculture Algérienne. Il serait souhaitable également d'étendre cette étude à de nombreuses autres variétés et lignées constituant la collection d'EL-KHEMIS (MILLANA).

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15. ZACHARIAS, M.

ABSTRACT

The okra-leaf shape was observed to induce higher grain yields when introduced into two cowpea varieties, Ife Brown and J33. Some of the differences in yield could be attributed to leaf area development and duration.

INTRODUCTION

Various researchers have reported that upright or vertical leaf orientation in cereal crops induced higher yields than other leaf orientations. Similar reports of this nature are not common in dicotyledonous plants. However, Andries et al. (1969) reported that the okra leaf character when introduced into cotton caused significant increase in yield and quality of lint.

The okra (hastate) leaf of cowpea is similar to that of cotton. It is characterized by very lanceolate trifoliolate leaves. The trait is determined by a single gene pair without dominance (Ojomo, 1977). The mutant is bushy and of no economic value but the okra leaf trait had been introduced into Ife Brown (a commercial variety) and J33 (an elite variety).

This paper reports the comparative yields obtained between normal plants and plants with okra leaves as well as the effects on growth and development.
MATERIALS AND METHOD

In 1976, one normal and one okra leaf population of J33 were compared using small 20-plant plots. Similar populations for Ife Brown were included in two more trials during 1977.

Plot size consisted of six 5m rows spaced 0.6m apart, 0.3m within rows and replicated five times. Two weeks after planting, two randomly selected plants were cut at ground level from each plot for growth analysis. Sampling continued bi-weekly till harvest. At each sampling time, plants were separated into stem, leaves and fruits (including flowers). Leaf area was determined by sub-sampling with cork borers. Dry weights were recorded after drying for 48 hours at 80°C.

Seed yield was taken at maturity. All data were run through a computerized two-way analysis of variance.

RESULTS

GROWTH HABIT

The data on some agronomic qualities are presented in Table 1. Though the leaf structure of the two varieties had been altered, the agronomic characteristics of the varieties were maintained by the selection pressure applied. However, the plants with okra leaves tended to trail more than the normal genotypes. Further, they were unattacked by Wet blight which was rampant in the early season of 1977.

At first sampling, two weeks after planting, the normal plants produced significantly higher leaf area than those with okra leaves (Table 2). The differences disappeared between four to six weeks after planting. Thereafter, plants with okra leaf produced higher leaf area than normal. Leaf area index followed the same trend as leaf area. The maximum leaf area index of between 2.04 and 2.94 was attained at 10 weeks after planting.

In all cases, dry weights (both total and of plant parts) increased with sampling time, reached a peak at 10 weeks and started to decline thereafter (Fig. 1). Between 6 - 8 weeks after planting, flowers had started to appear. Fruit weight increased and reached a maximum at 10 weeks and thereafter declined (Fig. 2).

For the first 6 weeks, the normal plants produced higher total dry matter (Fig. 1) but both types of plants partitioned dry matter similarly
between stem (30%) and leaves (70%). As growth continued, the proportion of total dry matter in the stem gradually increased from approximately 30% to 50% (Fig. 3).

From the 8th week till maturity when senescence occurred, plants with okra leaves produced more total dry matter than the normal ones. However, in Fig. 3 more dry matter was mobilized into fruits earlier by the normal plants than those with okra leaves (16.4 and 21.6% for H62 and J33 compared with 14.3 and 4.6% for their respective okra leafed counterparts). At maturity, there was no significant difference between the proportions of total dry matter stored into the fruits (Table 1, Harvest Index).

GRAIN YIELD

The yields of cowpea varieties with normal and okra leaves are given in Table 4. In the 3 experiments conducted under different climatic conditions, plants with okra leaves yielded significantly higher than the normal plants. The difference in yield between growing seasons is mostly due to weather conditions, particularly rainfall.

DISCUSSION

The yield advantages attributable to okra leaf plant types are similar to those reported by Andries et al. (1969). They attributed the improved yield to better light within the plant canopy leading to less boll rot and consequently increased yield. It was not possible to quantitatively measure the amount of light entering the cowpea canopy but it was obvious that more light reached lower leaves in the okra leafed plants than the normal ones. Similarly, less incidence of Wet blight disease was observed.

The differences in leaf area and leaf area index between the normal and okra leafed plants are similar to differences in total dry matter production and relative growth rates (i.e. amount of dry matter produced per sampling period). The values were significantly higher for the okra-leafed plants than the normal, especially at later stages of growth. The decline in dry matter during the last sampling period was due to loss of leaves arising from senescence.

Initially, the normal plants appeared more efficient in storing dry matter in fruits, but at maturity there were no significant differences in
harvest index. Therefore, the superiority in yield of the okra-leaflad
plants must be due to the increased bulk as shown by the total dry matter,
leaf area and leaf area duration. Similar observations had been recorded
in soybean by Enyi (1973) and Tayo (1977). Each found that leaf area
development was important in dry matter production and accumulation.
Further, Tayo (1977) observed that the soybean variety Hampton was highest
yielding because it had the highest leaf area, branches and nodes.

Plant architecture is very important in the cowpea crop.
Experience in Nigeria indicates that lots of flowers are produced that
never form pods (Ojehomon, 1969). Further, only 35-40% of the total
number of peduncles numbering 24-40 carry pods (Ojomo and Raji, 1976).
Yet they still grow to maturity, constituting a large part of the stem
component of the plant.

The maximum leaf area index obtained is 2.94 which is much less than
4-8 reported for cereal crops like maize and rice. All these factors no
doubt contribute to the relatively low yield of cowpea compared to other
crops. In the trial being reported, introducing hastate gene into
established background resulted in increased total dry matter production
without adverse effect on its distribution into plant parts. Increased
seed yield was observed. Similar restructuring may also prove advantageous
and are worth investigating.

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Table 1: Some growth characters of cowpea varieties with normal and okra leaves.

<table>
<thead>
<tr>
<th>Variety</th>
<th>% Germ.</th>
<th>Days 1st flower</th>
<th>Days 50% ripening</th>
<th>Shelling %</th>
<th>Harvest Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>H62XJ2-4</td>
<td>100.0</td>
<td>44.3</td>
<td>70.0</td>
<td>73</td>
<td>0.36</td>
</tr>
<tr>
<td>H62</td>
<td>100.0</td>
<td>43.0</td>
<td>72.0</td>
<td>76</td>
<td>0.41</td>
</tr>
<tr>
<td>J33XJ2-12</td>
<td>99.8</td>
<td>44.8</td>
<td>69.5</td>
<td>71</td>
<td>0.30</td>
</tr>
<tr>
<td>J33</td>
<td>95.8</td>
<td>44.0</td>
<td>70.8</td>
<td>73</td>
<td>0.36</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>3.3</td>
<td>1.6</td>
<td>1.7</td>
<td>6</td>
<td>n.s</td>
</tr>
</tbody>
</table>

n.s = Not statistically different at 5% level

Table 2: Leaf area (x1000 cm²) of cowpea with normal and okra leaves

<table>
<thead>
<tr>
<th>Weeks after planting</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>H62XJ2-4</td>
<td>0.05</td>
<td>0.50</td>
<td>3.13</td>
<td>5.47</td>
<td>4.54</td>
<td>1.72</td>
</tr>
<tr>
<td>H62</td>
<td>0.08</td>
<td>0.55</td>
<td>4.17</td>
<td>4.45</td>
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<td>1.25</td>
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<tr>
<td>J33XJ2-12</td>
<td>0.06</td>
<td>0.53</td>
<td>3.09</td>
<td>6.19</td>
<td>6.53</td>
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<tr>
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<td>4.06</td>
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<td>0.67</td>
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<tr>
<td>LSD 0.05</td>
<td>0.03</td>
<td>0.20</td>
<td>0.99</td>
<td>1.40</td>
<td>1.63</td>
<td>0.99</td>
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</tbody>
</table>

215
Table 3: Leaf area index of cowpea with normal and okra leaves.

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<th></th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
</tr>
</thead>
<tbody>
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<td>H62J2-4</td>
<td>0.23</td>
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<td>2.27</td>
<td>2.04</td>
<td>0.74</td>
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<td>2.01</td>
<td>0.99</td>
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</tr>
<tr>
<td>J33xJ2-12</td>
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<td>2.79</td>
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<tr>
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<td>1.65</td>
<td>1.05</td>
<td>0.30</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>0.08</td>
<td>0.46</td>
<td>0.76</td>
<td>0.75</td>
<td>0.35</td>
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</table>

Table 4: Mean grain yield of cowpea varieties with normal or okra leaves.

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<tr>
<th></th>
<th>1976 late*</th>
<th>1977 early</th>
<th>1977 late</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kg/Plot</td>
<td>Kg/ha</td>
<td>Kg/ha</td>
</tr>
<tr>
<td>H62xJ2-4</td>
<td>-</td>
<td>838.0 a</td>
<td>672.2 a</td>
</tr>
<tr>
<td>H62</td>
<td>-</td>
<td>531.0 b</td>
<td>366.7 b</td>
</tr>
<tr>
<td>J33xJ2-12</td>
<td>0.56 a</td>
<td>770.8 a</td>
<td>627.8 a</td>
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<tr>
<td>J33</td>
<td>0.39 b</td>
<td>236.3 c</td>
<td>455.6 b</td>
</tr>
<tr>
<td>SS</td>
<td>0.11</td>
<td>124.2</td>
<td>133.3</td>
</tr>
</tbody>
</table>

* 1976 data were from microplots.

* Values sharing the same letters within same column are not significantly different at 5%.
ETUDE COMPAREE DES EFFETS DES RAYONS GAMMA ET DES NEUTRONS RAPIDES SUR LE SOJA.

B. OUGOUA et I. NICOLAS
Institut National Agronomique
El-Harrach -ALGER- ALGERIE -

Abstract

Following promising trials with introduced soybean mutants from Roumania, a project on mutation breeding was started using a Chinese and a Roumanian variety as base material. The radiation treatment was carried out by the IAEA Laboratory Seibersdorf. First results of the experiments are reported.

Introduction:

L'utilisation des réacteurs atomiques, les études entamées depuis plus d'une vingtaine d'années, tant du point de vue fondamentale que sur le plan appliqué à l'amélioration des plantes ont mis en évidence l'efficacité mutagène des neutrons par rapport aux rayons gamma (8). Mais la spécificité des différentes radiations quant à l'induction (dans de fortes proportions) de tel ou tel type de mutation est loin d'être bien établie.

Aussi, le choix des doses d'irradiation pour l'induction d'une efficacité mutagène relativement élevée est souvent controversé par plusieurs auteurs (7).

Ainsi par notre étude comparée des effets des rayons gamma et des neutrons rapides sur le soja en première et deuxième génération nous essayons d'apporter notre contribution à l'élucidation de ces questions.

MATERIEL et METHODES:

Deux variétés de soja (Glycine Max L. Merrill) ont été utilisées dans notre étude, la Kai-yu 3 variété très ancienne (originaire de chine) et la B 79/35 (originaire de Roumanie) lignée pure issue d'une mutante irradiée qu'elle même est une mutante obtenue dans la variété américaine chippewa après irradiation aux rayons gamma.

Des 1000 grains de chacune des deux variétés ont été irradiés à 5 doses de rayons gamma (10-20-30-40-50 Krads) et également 5 doses de neutrons rapides (2,0-2,5-3,0-3,5-4,0 krads).
Les grains irradiés ont été semés à l'époque optimale en plein champ et disposé selon la "méthode des paires". À la maturité complète toutes les plantes de la première génération ont été récoltées individuellement dont les grains sont semés en plein champ et suivant la méthode un "plant par ligne".

Au stade levée de la deuxième génération (M₂) nous avons faits des comptages des nombres de plantes ayant présenté des mutations chlorophylliennes, et exprimé ces nombres en pourcent du nombre total des plantes levées par dose de chaque traitement.

Pour l'établissement du spectre des phénotypes des mutations chlorophylliennes, nous utilisons la classification proposée par GUSTAFSSON(1940) (4).

Afin d'apprécier l'efficacité mutagène des différentes doses administrées, nous avons utilisé la méthode proposée par KONZAK et colab(1964)(4) qui est basé sur le calcul des rapports MCP/L, MCP/S, MTP/S, MTP/L, MP/L et MP/S où MCP = fréquence des mutations chlorophylliennes en 0/00 des plantes M₂.

MTP = fréquence des mutations totales (chlorophylliennes et morphophysiologiques exprimée en 0/00 de plantes M₂.

MP(∗) = fréquence des mutations raccourcissant la durée du cycle végétatif, exprimée en 0/00 des plantes M₂.

L = létalité exprimée en % des plantes M₁.

S = stérilité exprimée en % des plantes M₁.

RESULTATS et DISCUSSIONS:

Les figures 1 et 2 montrent que les neutrons rapides pour une même survie provoquent plus de stérilité que les rayons gamma en première génération. Le tableau No1 confirme les résultats déjà obtenus (8) relatifs à l'efficacité des neutrons pour produire une plus grande variabilité.

Le tableau No2 présente les taux et les fréquences d'apparition des principaux phénotypes de mutations chlorophylliennes calculées en pourcentage des nombres de plantes levées pour les différentes doses de chaque traitement. Ce tableau fait apparaître une dépendance assez forte entre les taux de mutations et les doses d'irradiation, ceci est bien mis en évidence dans les figures 3 et 4.

(*) Ce terme MP des rapports MP/L et MP/S n'est pas prévu dans la méthode de Kozak. Nous avons jugé utile de l'ajouter car la précocité est l'objectif principal visé dans la sélection du Soja en Algérie.
Nous remarquons une efficacité relative des neutrons rapides dans l’induction des mutations chlorophylliennes par rapport aux rayons gamma. Le tableau N°3 représentant le spectre des quatre phénotypes des mutations chlorophylliennes identifiées, ne fait pas ressortir une spécificité mutagène des rayons gamma et des neutrons rapides à l’égard des principales mutations chlorophylliennes comme il a été démontré par plusieurs auteurs tels que GAUL.H (1964) et D’AMORO.F, SCARASCHI.A.G (1961) (8). Mais on ne peut cependant conclure à l’inexistence de ce phénomène qui peut-être mis en évidence par une étude individuelle du point de vue génétique et cytogénétique d’un grand nombre de mutations chlorophylliennes viables induites par les différentes radiations ionisantes. En effet selon P.PERBAU LEROY (1968) chaque phénotype de mutation chlorophyllienne est contrôlé par un grand nombre de loci; si quelques uns d’entre eux seulement ont une sensibilité particulière à une radiation donnée, celle-ci ne peut apparaître dans une analyse globale.

![Graphique](image.png)

**Fig. 1.** Relation entre la survie et la stérilité des plantes sous l’action de l’irradiation, chez la *C. caryophyllacea.*
Fig. 2. Relation entre la survie et la stérilité des plantes sous l'action de l'irradiation, chez la Rauvou 3.
**Effet de l’irradiation sur quelques caractères morphologiques.**

**Tableau 1**

<table>
<thead>
<tr>
<th>Traitement</th>
<th>Dose (Kra/ls)</th>
<th>Pourcentage de Plantes présen-</th>
<th>Pourcentage de Plantes présen-</th>
<th>Pourcentage de Plantes présent-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>tant les défis</td>
<td>chloro-phylliennes</td>
<td>des feuilles/des tiges</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variétés</td>
<td>Variétés</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B79/35</td>
<td>Kaï-yu 3</td>
<td>B79/35</td>
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<tr>
<td><strong>Non irradié</strong> (T)</td>
<td></td>
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<tr>
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<td>56,1</td>
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<td><strong>Neutrons rapides</strong></td>
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<td>100</td>
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<td>Traitements</td>
<td>Dose (Krd)</td>
<td>Fréquence des Mutations (%)</td>
<td>Taux de Mutations</td>
<td></td>
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<tr>
<td>------------</td>
<td>------------</td>
<td>-----------------------------</td>
<td>-------------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B 79/35</td>
<td>Kaï-yu N°3</td>
<td>B 79/35</td>
<td>Kaï-yu N°3</td>
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<td>Radiations</td>
<td></td>
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<tr>
<td>Gamma</td>
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</table>
### Spectre des Mutations Chlorophylliennes sur M_2

**Tableau: 3**

<table>
<thead>
<tr>
<th>Traitement</th>
<th>Dosage (Krad)</th>
<th>Albina (%)</th>
<th>Xantha (%)</th>
<th>Dark-Xantha (%)</th>
<th>Chlorina (%)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>**D79/35</td>
<td>Kaf-yu3**</td>
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<tr>
<td><strong>Radiations</strong></td>
<td></td>
<td></td>
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<tr>
<td>Gamma</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<td></td>
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<td>18,6</td>
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</table>
Fig. 3 - Relation entre la fréquence des mutations chlorophylliennes et les doses d'irradiation chez la \( \mathbf{B} \ 79/85 \).
Nous avons regroupé dans le tableau N°4 les résultats des calculs de l'efficacité mutagène des doses de rayons gamma et des neutrons rapides administrées. Nous remarquons dans ce tableau, que pour avoir une grande fréquence de mutations précoces avec un faible pourcentage de léthalité, les doses conseillées sont: pour la variété B79/35, 10 krads de rayons gamma, 3,0 krads de neutrons rapides. Pour la Kai-yu 3, 10 krads de rayons gamma, 2,5 krads de neutrons rapides.
Si nous désirons une grande fréquence de mutations totales avec peu d'effets néfastes (létalité, stérilité) les doses recommandées pour un pourcentage de létalité faible sont:
chez la B79/35, 10 krad de rayons gamma, 3,0 krad de neutrons rapides,
chez la Kai-yu 3, 10 krad de rayons gamma et 2,0 krad de neutrons rapides.

L'examen globale de ce tableau met en évidence l'efficacité de faibles doses chez les deux types de rayonnements et pour les deux variétés. Ainsi toutes les doses provoquant une bonne efficacité relative, sont situées au dessous de la DL-50 et nous appuient les thèses avancées par Delone (1966) et Enken (1967) (7) en ce qui concerne l'intérêt de l'utilisation des doses faibles et modérées.

CONCLUSION :

La comparaison des effets des radiations utilisées, fait apparaître une plus grande efficacité des neutrons rapides par rapport aux rayons gamma. Aussi par l'établissement de la fréquence d'apparition des mutations chlorophylliennes et de leur spectre, nous n'avons pu mettre en évidence une spécificité mutagène des rayons gamma et des neutrons rapides à l'égard des principales mutations chlorophylliennes comme il a été démontré par plusieurs auteurs. Évidemment nous ne pouvons conclure à l'inexistence de ce phénomène, qui peut-être mis en évidence par une étude détaillée au point de vue cytogénétique et génétique.

Les calculs de l'efficacité mutagène des doses des radiations gamma et des neutrons rapides mettent en évidence l'efficacité des doses faibles et modérées. Ainsi pour l'obtention d'une fréquence élevée de mutations précoces avec un faible pourcentage de létalité, nous préconisons les doses suivantes 10 Krad de rayons gamma pour les deux variétés traitées et respectivement 3,0 Krad et 2,5 Krad de neutrons rapides pour la B 79/35 et pour la Kai-yu 3.

Toutes les doses pour lesquelles nous avons eu les plus grandes fréquences de diverses mutations sont en général plus petites que les DL-50. Nous confirmons par ces résultats les thèses avancées par certains chercheurs conseillant l'utilisation des doses faibles et modérées.

Au terme de la comparaison des réactions des deux variétés traitées, nous tenons à souligner que la lignée père B 79/35 (obtenue par l'irradiation d'une lignée qui elle-même est une mutante issue de la variété américaine Chippewa après irradiation) a manifesté une plus grande radiosensibilité et a donné plus de variabilité par rapport à la Kai-yu 3 (ancienne variété très stable). Il en résulte donc que l'irradiation répétée peut-être une méthode très efficace pour l'obtention d'une grande génétique.
<table>
<thead>
<tr>
<th>Variétés</th>
<th>Traitement</th>
<th>Dose (Kv)</th>
<th>Mutations en % de plantes Mg.</th>
<th>Effets nocifs sur la Mg.</th>
<th>Eficiencia Mutagène</th>
<th>MCP/L</th>
<th>MCP/S</th>
<th>MP/L</th>
<th>MP/S</th>
<th>TCPF/L</th>
<th>TCPF/S</th>
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</thead>
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<td>Chlorophyl</td>
<td>Récesses</td>
<td>Survie en % (L)</td>
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<td></td>
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<td>liennes</td>
<td>M. P.</td>
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<td>Totales M. P.</td>
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<td></td>
<td></td>
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I. I. T. A. PROJECTS
BREEDING OF ROOT AND TUBER CROPS

S.K. Hahn, A.K. Howland and J.E. Wilson
International Institute of Tropical Agriculture (IITA)
Ibadan, Nigeria

ABSTRACT

The root and tuber crops cassava, yams and sweet potato are important in Nigeria and Africa. Breeding methods most appropriate to these crops are discussed in terms of mating systems, creation of source populations, selections. Intervarietal hybridization, interspecific hybridization, and population improvement through cyclic selection and recombination procedures are methods of creating source populations. Factors affecting the effectiveness of selection, and efficient methods of selection to make maximum genetic progress are discussed. Use of selections is briefly stated. Synthetics, composites, or single and double crosses of yam produced from true seed may be used at the farmer level.

INTRODUCTION

The important root and tuber crops in Nigeria and in Africa in general are cassava, yams, sweet potato and cocoyams. These are all vegetatively propagated crops.

Production of cassava in Nigeria amounts to about 10 million t annually from 1 million hectares, and the trend in production shows a steady increase (3). Cassava is potentially able to produce more food calories per unit area than any other lowland crop. It requires less management input than cereal crops and yams, and it can be available for harvest throughout the year. It has the potential for use as livestock feed and industrial production of starch and alcohol. Cassava is grown in a wide range of environments: annual rainfall ranging from 500 mm to 5,000 mm, and altitudes from sea level to 1,500 m, latitudes 30°N to 30°S, annual average temperature from 15°C to 35°C, soils varying from rich loamy to poor and acid sandy and management from minimal to relatively intense.

Nigeria produces about 15 million t. of yams annually from 1.4 million hectares (3). White yam (Dioscorea rotundata), yellow yam (D. cayenensis) and cluster yam (D. dumetorum) which are indigenous to West Africa, and water yam (D. alata) which originated in Asia are the major food species. They grow well in lowland humid areas as well as derived savannah areas. In Nigeria yams have high market value, they give higher income than other root crops and they do not require the tedious processing and cooking procedures necessary for cassava.

Sweet potato is not very popular in Nigeria and only about 200 thousand t are produced annually from 15 thousand hectares (3). However, on the world scale, it is the sixth most important staple food crop, with 130 million t produced annually on 15 million hectares. In addition to its importance as human food, it provides animal feed and raw material for industrial purposes. Sweet potato is grown over a wide range of environments within latitudes 40°N and 40°S and at altitudes as high as 2,000 m above sea level. Considerable yields can be obtained from land of low fertility and relatively low pH (9), and although it is not as highly tolerant to drought as cassava, it does have good drought tolerance. Sweet potato requires relatively little attention and labour, and therefore production costs are low. In Western Nigeria, it is capable of producing about 30 - 40 t per hectare in four months, without fertilizers.

The major biological constraint to cassava production in Nigeria are diseases, especially cassava mosaic disease (CMD) and cassava bacterial blight (CBB). Most of the local cassava cultivars are susceptible and yields are low, being about 5-10 t of fresh yield per hectare in 12 months compared with potential yields of more than 20 t. Processing is labour intensive, however, there are few storage problems since it is kept in the field until used.
Yams are costly to produce because of the large quantity of planting material required and the high number of man-days needed for staking and harvesting. Staking materials are expensive and in some areas are not available. Yams are attacked by virus, leaf spot diseases and nematodes, and storage problems are many including heavy post harvest losses due to nematodes, fungal and bacterial rots, insect and rodent damage, and losses due to respiration and premature sprouting. Yams are normally grown in relatively rich soil; nonetheless, yields are low, about 11 t per hectare in 6 to 10 months. The rate of multiplication is low.

The major constraints is sweet potato production in Nigeria and in Africa are viruses and sweet potato weevil (*Cylas puncticollis*). Yield reduction from virus is about 80% (14) and from sweet potato weevil about 60% (12). Storage problems are: storage rot, insect damage especially by weevil, and physiological loss under ambient conditions.

Although cassava and yams are important in Africa and sweet potato has tremendous potential, improvement of these crops has been given little attention in the past. The International Institute of Tropical Agriculture (IITA) established the Root and Tuber Improvement Programme in 1971. This programme covers cassava, yams, sweet potato and cocoyams in that order of priority. The broad objectives are to develop improved cultural practices and varieties with high, stable yield, high quality, and plant characteristics suitable for efficient cropping systems. To achieve these objectives the programme involves agronomy, breeding, entomology, pathology, physiology and bio-chemistry.

Significant progress has been made in cassava by producing improved clones with resistance to disease (especially CMD and OED), high yield, improved root characteristics, low HCN content, and resistance to lodging. New high yielding sweet potato clones have field resistance to viruses and weevil, and yield potentials of about 30 or more t of fresh yield in four months, without fertilizers. In yams, techniques for germinating seed, controlled hybridisation, rapid multiplication using rooted vine cutting, and screening for resistance to yam viruses have been developed, and are used for the improvement primarily of white yam.

The success of the plant breeder depends in large measure on the efficiency of the techniques and methods he uses and the skill with which he employs them. It is not possible here to consider all the breeding procedures, only a few will be discussed.

Vegetatively propagated crops are characterized by the following advantages:

1. as long as they are propagated vegetatively, no genetic segregation takes place even if they are genetically heterozygous,
2. superiority of F₁ plants results from both additive and non-additive effects. Heterosis can be vegetatively fixed, it lasts permanently, and it is therefore possible to select individuals having desirable characteristics in the F₁ generation.

The disadvantages are:

1. many cultivars do not flower under natural conditions.
2. the inheritance of characters is usually very complicated due to heterozygosity and polyploidy.
3. many diseases and insects are transmitted through vegetative propagation, and
4. exchanging breeding materials is difficult because of local quarantine regulations governing the export and import of vegetative materials.
STEPS IN PLANT BREEDING

Plant breeding involves three major stages:

1. finding or developing the populations from which selections can be made,
2. selection, and
3. the use of selections in commercial production.

Source Population

Selection can be effective only where significant genetic variation exists. Furthermore, its effectiveness is related to frequencies of the desired alleles controlling the characters being selected.

In the early days, the breeder has collected germ plasm in vegetative form from farmers' fields and from natural stands within and outside the country. Much effort has been expended in searching in such populations for the chance occurrence of individuals having desirable characteristics. A better approach is to create new source populations. To do this the germ plasm is evaluated and the most promising clones are intercrossed. Parental selection is of prime importance. It should be based on their breeding values as determined by appropriate progeny evaluation but parents may also be selected by a geometrical approach without progeny testing (5). Source populations having larger genetic variation can be created by interspecific crossing with related species which contain unexplored and potentially useful genes. There is a need to develop methods for the introgression of exotic germ plasm into breeding populations.

Selection of sweet potato for source potential may be done by grafting breeding materials onto test varieties. Several clones with high source potentials were selected (14) and these will be used as parents to improve the photosynthetic capacity.

Hybridization of yams was reported in 1971 (10) and the method has been further applied with success (14, 15).

Population improvement using cyclic recombination and selection procedures is an effective approach to increase desired gene frequencies while retaining a high degree of genetic variability. Cassava is monoecious, with female flowers at the base opening first and apical male flowers normally opening about 7 to 10 days later (7). Yams are normally dioecious with male and female flowers on separate plants. Sweet potato is normally self-incompatible. Therefore, all three crops are normally cross pollinated, cassava and sweet potato mainly by bees and yams by thrips (10). The flowering habits and mating system of these crops make population improvement schemes appropriate.

As we look ahead to the next decade, we are convinced that population improvement will be successful in creating improved source populations and continuously upgrading these populations. Various methods used in breeding systems for population improvement were summarized by Gardner (4) as follows:

Inter-population improvement methods:
- Mass selection
- Half-sib family selection
- Full-sib family selection
- S1 family selection

Inter-population improvement methods:
- Half-sib reciprocal recurrent selection
- Full-sib reciprocal recurrent selection.

An appropriate method of population improvement should be chosen from those listed above. Major genes that affect qualitative traits are relatively easy to substitute into populations and their effects are easily identified. However, substitution is difficult for the so-called polygenes that affect characters like yield and which are the ones we particularly hope to improve by population improvement. It was proposed by Hahn et al. (7) that in cassava half-sib family selection would be the most appropriate. This method may also be the most appropriate for yams and sweet potato.
The authors have been using the population improvement method with great success for breeding improved cassava and sweet potato, and it is now being applied to yam improvement. Cassava from Latin America and India, which are susceptible to CMD, CBB and lodging but have other desirable agronomic traits were crossed twice with local sources and selections subsequently made. In this manner, these exotics have been substantially improved for resistance to CMD and CBB and to lodging, while maintaining the other desirable traits (14).

Selection

Genetic progress \( G \) is a function of the selected differential \( D \) and heritability \( H \) and is expressed by \( G = D \cdot H \) (16). For characters of high heritability, effective progress can be made by phenotypic selection. For characters of low heritability, selection must be based on genotype determined through progeny testing. In the cross-pollinated root and tuber crops, the most appropriate progeny test appears to be one in which is based on the progeny of crosses, i.e. combining ability test. Combining ability can be differentiated into "general" and "specific" (18). A logical procedure is to evaluate clones first for general combining ability using test-crosses in which the tester parent provides a wide range of genotypes with respect to the characters for which selection is being made. As a second step, the lines found superior in general combining ability may be tested for specific combining ability.

General and specific combining abilities of six cassava cultivars were investigated (12). Clone 58308 had high general combining ability for resistance to CMD, CBB, and for yield and HCN. Analysis of variance for general and specific combining abilities indicated that additive parental contributions accounted for most of other variation, although significant non-additive effects were present for most of the characters, except for CMD and yield. It was observed from a 6 x 6 parental diallel cross of sweet potato that Tib 8 and Norin 20 had high general combining ability for yield in three environments (13). Tib 6 and Norin 20 had high general combining abilities for resistance to viruses (13). From an 8 x 8 diallel cross of sweet potato, Tib 2525 showed the largest general combining ability for resistance to tuber damage by weevil followed by local cultivars Tib 6 and Tib 5 (15).

In cassava, resistance to CMD has a high heritability of about 60% (12), and heritability of about 40% was obtained for resistance to CBB, for tuberous root size and shape and for neck length (13). In sweet potato, heritabilities for yield, average number of tuberous roots per plant, and average tuberous root size were larger in the rainy season than in the dry season (45%, 45%, 55% respectively compared with 35%, 25% and 36%) (11). The rainy season is, therefore, more favourable than the dry season for evaluating sweet potato breeding clones or varieties. From a 6 x 6 diallel cross, a high heritability of 84% for resistance to virus of sweet potato was established (13). Heritability of resistance to weevil in terms of tuber damage was only 5% (15).

The genetic relationship between characters can affect the genetic progress because selection for one character may favour or be at the expense of another character. It was reported that there exists in cassava a highly significant genetic correlation between resistance to CMD and CBB (6, 12, 13, 14, 15). Dry matter percent in cassava was found to be significantly related to starch content with \( r = 0.81^{**} \) for 205 clones (13). In white yam, yield is associated with sex, namely female and monocious plants outyield male plants, and male plants out-yield non-flowering plants (14, 15). Yield of sweet potato was found to be significantly positively associated with average number of tuberous roots \( (r = 0.60^{**} \) but not significantly associated with average tuberous root size \( (r = -0.3 \alpha 0.2) \), and average number of tuberous roots per plant was significantly negatively associated with average tuberous root size \( (r = -0.5) \) (11). Therefore, selection for large number of tuberous roots will results in smaller tuber size and vice versa. High genetic correlation between tuber damage and tuber depth \( (r = 0.83^{**}) \) was obtained, which was due to both additive and non-additive effects (15).

Understanding the inheritance of characters is also important in laying down breeding strategies, but to date there is not much information available regarding the agronomically important characteristics of roots and tubers. In cassava resistance to CMD is regulated by quantitative genes with additive effects and appears to be recessive (6, 12). Resistance to CBB
also appears to be due to quantitative genes mainly with additive effects but also to some extent with non-additive effects and it appears to be recessive (8). Low HCN content is regulated by a recessive minor gene complex (12).

In the root and tuber crops sufficient seed for a progeny test can be obtained by a polycross. The polycross test as a method of evaluating clones for general combining ability is cumbersome when large numbers of selections have to be evaluated, and an open-pollinated progeny test should be used for preliminary screening.

Plant-row selection as proposed by Lonquist (17) involves half-sib family selection. This half-sib family system, combined with individual plant selection within families, has given excellent results for improving yield of corn (19). Selection is based on half-sib family means, and subsequently on individual phenotypes within selected families. This method should be effective if trials are conducted in 3 to 4 locations using a small plot size and only 2 replications per location. Plant-row selection requires only one generation per cycle, no hand pollinations are necessary, and it includes:

1) yield trial evaluation between progenies,
2) individual plant selection within progenies and
3) recombination.

Large numbers can easily be handled. The plant-row selection could make possible more rapid accumulation of favourable genes or gene complexes of the additive sort without excessive inbreeding and it permits the retention of much of the favourable genetic material present in the original gene pool.

About 50 cassava families have been selected each year based on origin, resistance to CMD and CBB, conformation, root characteristics and yield potential. They were planted each in rows, in two replications, in an isolated plot. Bulked root stocks of these families were interplanted to provide pollen, discarding those having undesirable root characteristics and root rot. Male flowers on the female plant rows were removed to prevent inbreeding within the plant-row. Selection among families was made, based on average performance in subsequent trials, and also selection of desirable individuals within the selected family was practised. Using this system, many promising selections of cassava have been made (15).

Constant selection pressure based upon reliable evaluation procedures should bring about considerable modification of the population in the direction desired. When the remaining genetic variance is largely dominant and epistatic, clones for F₁ hybrid combination can be extracted.

It has been suggested that the comprehensive breeding scheme proposed by Eberhart et al. (2) be applied to cassava breeding (7). Two cassava sources, one from Nigeria and the other one from Latin America were random mated. Each source was composited into a population and permitted to intercross within itself for two generations without selection, although natural selection for resistance to cassava bacterial blight took place. In 1976 the two populations were planted in an isolated plot and intercrossing between the populations was permitted in order to introgress new germ plasm and to provide maximum opportunity for genetic recombination. This cycle will be repeated applying some selection pressure and introducing additional new sources into each population. By using this scheme the frequency of low leaf HCN plants was increased by 10% in both the local and exotic composite populations (15).

Hahn et al. (7) have discussed population size, number of generations and selection pressure appropriate for cassava. The size of population that should be saved at each cycle of improvement will depend on the selection scheme, variation desired, funds and land space available. About 500-1,000 plants of root and tuber crops should be sufficient to prevent loss of favourable genes due to finite population size. Two or three generations of random mating should be used to achieve synthesis in a population before initiating selection (4). Selection pressure and stage of selection should vary with the characters to be improved. In the first cycle about 5-10% selection pressure and in the second and third cycles 25-50% can be applied.

To ensure adaptation of improved varieties and to evaluate genotypes effectively, we require information on genotype-environment interaction. In cassava,
fresh yield showed highly significant variety x spacing, variety x season, and variety x year interactions, and dry matter showed high variety x season interaction (14). Resistance to CMD which had been selected in Nigeria has been found to be stable in other countries i.e. Zaire, Cameroon, Sierra Leone, Liberia and India (15). Resistance to CBB tested in Nigeria was confirmed in Zaire where the disease is also very serious (14). In sweet potato, correlation of yield estimated in rainy and dry seasons was high (11). Genotype x season interaction was highly significant for fresh yield, tuberous root number and tuberous root size of sweet potato (12). Testing cassava varieties for two years during two seasons, and at two spacings gave the smallest standard error, indicating that this testing method will give the highest precision in yield trials of varieties (14). An analysis of yield data from an 11-cultivar yield trial revealed that a 4-row plot, 4 x 12 m, with four replications, harvesting only the center 2 x 10 m plot was the most satisfactory for precision, land use and expense (12, 14).

Use of Selection

Yam setts are expensive, they often transmit diseases such as virus and nematodes, and multiplication of sett yams is slow. In the future these limitations may be overcome by developing homogeneous varieties, synthetics, composites or hybrids to be grown from true seed at the farm level. Even if two years are required to produce ware yam from true seed, i.e. seeds to setts, the first year, setts to ware yam the second year, the advantages of using true seed may be substantial.

In the development of synthetics the following points should be kept in mind:

1. parental lines or clones should be selected with the appropriate combining ability test for measuring potential performance in synthetics,
2. only a small number of well selected clones should be used,
3. the yields from F₁ to advanced generations should not decline rapidly and
4. such characters as tuber yield, size, shape and culinary qualities should be uniform.

It may be possible to use true hybrid seeds of promising single or double cross combinations. Use of double cross may not be advantageous in yams since many seeds may easily be obtained and the vegetatively propagated parents can be multiplied rapidly by rooted cuttings or tissue culture.

Synthetics or composites can be improved through recurrent or reciprocal recurrent selection, by selecting clones whose polycross progenies rank in the upper 10% for yield. Thus synthetics or composites from advanced cycles of recurrent selection should outyield the first cycle. It is generally believed that the additive fraction of genetic variance can be made use of in synthetics but if substantial non-additive variance is to be exploited, first generation hybrids must be used.

The improved seed of cassava, yams and sweet potato developed through population improvement can be distributed to research institutions in other countries for establishing source population for their breeding programmes. Distributing breeding material in seed form will face less quarantine restriction and carry less risk of distributing diseases and pests. Genotypes best adapted to a given environment can then be selected on site thus reducing the problems of adaptation, multiplication, and distribution.
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RECENT PROGRESS IN VARIETAL IMPROVEMENT OF COWPEA AT IITA

J.B. Smithson
International Institute of Tropical Agriculture
Ibadan, Nigeria

ABSTRACT

Cowpea, (Vigna unguiculata L. Walp) is an important component of cereal cropping systems from the semi-arid to humid tropics. In West Africa, cultivars are locally adapted through photoperiod response but yields are low primarily due to insect and disease problems.

Five interfertile subspecies are recognised, but attempts of hybridization with other species have been unsuccessful. Cowpea was probably domesticated in West Africa and centres of diversity occur in West Africa and India.

The cowpea breeding programme at IITA and the manner in which it is developing are described.

INTRODUCTION

Cowpea (Vigna unguiculata L. Walp) utilised as grain, as leaf or green pod vegetable or as forage, is an important component of farming systems from the semi-arid to humid tropics, providing a protein supplement in diets primarily carbohydrate in nature and with a range of diversity which suits the crop to a wide variety of situations. It is chiefly important as a grain in the savanna areas of West Africa and South America where it is grown in association with the cereals - millet, sorghum and maize - in traditional agricultural systems.

In West Africa, both sorghum and cowpea are photoperiodically adapted to the localities in which they are grown so that they flower at the end of the rainy season and mature their grain on residual soil moisture under the relatively favourable conditions of the early dry season. However, neither fertilizer nor insecticide is applied, plant populations are sparse and yields are low - of the order of 200 - 300 kg grain per hectare. In more humid regions, cowpea is grown for green pod vegetable utilization. In most situations, but particularly in most humid tropics, the main limiting factors to production are insects and diseases.

ORIGIN AND EVOLUTION

The origins and evolution of cowpea have recently been reviewed by Steele (1978). Five subspecies are recognized (Verdcourt, 1970). These are:

Subsp. unguiculata, the cultivated cowpea of the African savannas.
Subsp. cylindrica, the catjang, a fodder and grain type from India.
Subsp. *sesquipedelis*, the yard, long or asparagus bean of India, Southeast Asia and China, the green pod vegetable type. 
Subsp. *dekindtiana* and *mensensis*, the wild and weedy forms of Africa, putative progenitors of the cultivars.

Centres of genetic diversity occur in Africa and India (Steele, 1978). The current view is that subsp. *unguiculata* originated in Africa from the wild perennial subsp. *dekindtiana* and *mensensis*, probably by way of an annual wild form (Rawal, 1975). Subsp. *unguiculata* then followed trade routes to India more recently than 1500 B.C. and subsp. *cylindrica* and *sesquipedelis* evolved from it in India and South East Asia, respectively. The crop was established in Southern Europe by the end of the Roman era and reached the West Indies and the Southern USA in the 17th and 18th centuries (Wight, 1907).

The subspecies are interfertile and easy to hybridize. Rawal (1975) has presented evidence for the occurrence of gene exchange in nature between weed forms and cultivars in West Africa. However, successful hybridization between cowpea and other *Vigna* species has not yet been reported.

Thus, the current status of cowpea, its cultivation preponderantly in primitive agricultural systems, the occurrence of wild and weedy populations with which gene exchange is occurring and restricted breeding effort, all contribute to the very striking range of diversity which is available for exploitation in varietal improvement programmes.

THE IITA PROGRAM

A. Objectives

Major emphasis is placed on the incorporation of disease and insect resistance into a range of plant types and photoperiod reactions suited to different cropping systems and environments and making the resulting lines available to national cowpea programmes.

B. Early progress in varietal improvement

The development of the cowpea breeding programme of the International Institute of Tropical Agriculture has been documented in Annual Reports (IITA, 1971; 1972; 1973; 1974; 1975) and described by Rawal (1975), Rachie and Rawal (1976) and Smithson et al. (1978).

The breeding procedures utilised may be considered under three main headings:
(a) Germplasm collection and evaluation

By the end of 1975, the IITA cowpea germplasm collections comprised over 7,300 accessions of *Vigna unguiculata* and 145 other *Vigna* species (Rawal, 1975). Almost 6,000 of these have been evaluated for up to 46 plant characters and information relating to the first 4,224 accessions has been published (IITA 1974b). Resulting from this work variation has been identified for many important plant characteristics. The Information Sciences/Genetic Resources Unit of the University of Colorado are using cluster analysis techniques to analyse the variation (Rawal et al. 1977) and identify duplicates (Bryant et al. 1977). In addition, four lines combining good yield performance with resistance to a range of diseases and insects (IITA, 1975, 1976) and a fifth, comprising an $F_2$ population segregating for male sterility (IITA, 1975) have been described as VITA lines.

(b) Conventional breeding

The identification of suitable breeding materials was accompanied by the intensification of recombination procedures and by 1975, 3,000 crosses had been achieved using conventional crossing methods. Comparisons of lines emerging from this program across environments (Nangju et al.; 1975; 1977; IITA, 1976; 1977) indicate a progressive improvement in yield level in the breeding material relative to the VITA lines. It is notable that many of the higher yielding lines now emerging exhibit the hastate or narrow leaf shape indicating a real advantage for this characteristic (IITA, 1976; 1977). Also notable are the large and significant interactions which occur between varieties and environments. Estimates of location, variety, location x variety and error variances for seed yields obtained from three series of trials of advanced breeding lines in 1977 indicate that location and variety x location effects are in every case very highly significant and much larger than the variety components. The linear regression of individual variety on location mean yields accounts for the greater proportion of the interaction which is therefore to some extent predictable, but analysis of these and other data is in progress to obtain a clearer understanding of the factors contributing to variation in yields and the interaction between varieties and environments.
(c) **Population improvement**

Conventional breeding methods, although they have contributed significantly to crop improvement, especially with regard to characters controlled by relatively few genes, impose severe restrictions on genetic progress due to limitations on the numbers of parental materials which may be used to initiate a crossing series and the reduced probability of the formation of novel recombinants due to linkage (Jensen, 1970; Doggett, 1970; 1972). These restrictions may be substantially reduced by the adoption of the population improvement and recurrent selection methods which have been so successfully applied in cross-pollinated crops. The occurrence of a simply inherited recessive factor governing male sterility (Rachie et al., 1974), the ease of conventional crossing (Rachie et al., 1975) and its short generation interval make cowpea a highly suitable crop for the application of such procedures (Rachie and Gardner, 1975). Rachie and Gardner (1975) and Rachie and Rawal (1976) proposed the development of a system in which sub-populations selected for specific characters feed into a back-up and thence into a main population. Different elements of the system have now been assembled and synchronized to facilitate the interchange of materials. The main population is represented by a recurrent selection system operating with conventional crossing rather than male sterility.

Some evidence of changes in the populations has been obtained (Rawal, unpublished). Increased frequencies of genes for white colour of the seed coat and flower and resistance to *Cercospora* and anthracnose and a mean yield improvement of 17.6% over the original parents were demonstrated following one cycle of selection. Comparisons of selections from the subsequent cycle indicated no further trend in seed yields but some families yielded significantly higher than the populations from which they were selected and one line from the earlier cycle is currently in international yield trials. Additional advantages in terms of adaptation to different environments and interchange of genetic materials are indicated by further unpublished data from Fort Collins where although only 3 of 45 donor parents
flowered and set pods, 68% of the plants in a first cycle population reached maturity.

C. Recent developments

Since 1975 at IITA (IITA, 1976; 1977) a three tiered structure has evolved aimed at providing a range of breeding materials, from pure lines to segregating populations for breeders in national programmes. These involve backcross methods to transfer one or a few characters to otherwise superior backgrounds and recurrent selection procedures using on the one hand conventional crossing among improved lines and on the other, male sterility to generate wider variation. In each case, the short generation interval of cowpea and the Ibadan climate are exploited to advance generations at the rate of 3 or 4 per year.

To limit seed movements and thus the risk of dissemination of seed borne pathogens between regions, and yet to approach a solution to the problems of genotype x environment interaction a set of testing locations has been developed which takes advantage of the ecological diversity within Nigeria from the very humid tropics with 2,000 mm rainfall per annum occurring during 9 months to the dry sahel with 500 mm in 4-5 months (Figure 1). A similar series is also being established in Upper Volta and the nuclei of regional programmes in East Africa and South America are being formed.

Recurrent selection procedures have developed according to the general structure proposed by Rachie and Gardner (1975). The main population is represented by a system analogous to $S_2$ family testing with four generations per annual cycle but using conventional crossing rather than male sterility. Hybridization involving some 200 combinations carefully selected to combine particular characteristics, $F_1$ and $F_2$ generations are grown at Ibadan during the period September to June. $F_2$ populations are inoculated with the main diseases and receive minimum insecticide to allow selection for disease and insect resistance. Selection pressure is also exerted for seed type and size. $F_3$ lines are then screened at the series of locations in West Africa. Single plant selections are made in lines which perform well across locations
(environment non-specific) and at individual locations (environment specific). These are multiplied at Ibadan during the dry season, to produce disease-free seed, and become entries in preliminary yield trials in the following main season in West Africa and in other regions where facilities are available. Selected lines progress to advanced and international trials at an increasing number of locations in successive years preceded by dry season multiplication at Ibadan. All entries in the trials are sown as non-replicated plots at Ibadan for further single plant selection and are tested by the pathologist and entomologist in field and screenhouse for resistance to the major pests and diseases. Each year the cycle is reinitiated by recombination of selected lines from each stage and new materials from elsewhere contributing particular characteristics. Lines from each stage are also available for distribution to national programmes depending on their capacity.

In the back-up and sub-populations, genetic male sterility is utilised to generate and maintain more extensive variation using moderate selection pressures and combined $S_2$ family and male sterile plant selection. In the second season from August to November, when disease and pest pressures are high, fertile and male sterile plants are selected in the current population and new lines, from the previous cycle or elsewhere are crossed on to the selected male steriles. During the dry season, from December to March, fertile ($S_1$) and male sterile ($F_1$) rows are multiplied. In the first season, from April to July, when insect pollinator activity is high, seed of male sterile rows are sown as $F_2$'s and intermated by crossing fertiles onto male steriles, seed of which is used to sow the third generation ($G_3$) in the second season. Meanwhile, $S_2$ families from the disease and insect subpopulations go to the pathologist and entomologist, respectively for screening and from the back-up populations to yield trials in several environments and selected lines and others crossed into the $G_3$ to initiate the new cycle.

The system is flexible. Selection and introduction of new lines may occur in the $F_2$ using four generations and two cycles per
year. Materials are readily exchangeable between populations. Sub-
populations may be formed for other purposes such as intercropping
and local adaptation, currently in progress, or other stress
conditions such as low soil moisture or pH. The existing structure
may not continue to be appropriate. As progress is made in the
population and we learn more about the factors limiting cowpea
production in different environments the establishment of populations
combining characteristics for particular ecological conditions may
be a more suitable strategy.

Finally, it is necessary to consider the ways in which
materials being developed may be best employed to meet the
needs of national programmes. The main constraints have been the
low priority given to cowpea and the lack of trained manpower. This
situation is slowly changing and interest and programmes are
developing in several countries.

In the meantime, for West Africa, preliminary and advanced breedi-
ging lines selected on the basis of performance across the major
environments are being made available to those countries with the
capacity for inclusion in national yield trials together with
locally developed cultivars. Final reselection could be done in
the national programmes. Proven lines from different national
trials would then enter regional trials which could be stratified
to accommodate different ecological zones and would serve as sources
of materials for those countries without active improvement programmes.
IITA’s role would then be the provision of suitable new materials
to national programmes and assistance with the co-ordination and
summary of the regional trials.

Extension to other regions is more difficult. Ideally, regional
programmes operating in the same manner as that in West Africa would
be envisaged. However, the capacity at IITA to handle breeding
materials will probably continue for some time to be greater than
that of centres in other regions, and the distribution of interna-
tional uniform cowpea trials will continue accordingly. Particularly
in terms of disease and insect screening, determination of appropriate
strategies will await the results of preliminary and advanced
trials now being distributed to key centres in other regions.
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Fig 1: West Africa. Cowpea breeding trials in relation to mean annual precipitation:

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Conclusions and Recommendations

Introduction

Upon invitation by FAO and IAEA the seminar was organized at the International Institute of Tropical Agriculture, Ibadan, Nigeria. 43 scientists participated in the seminar, including 8 invited experts from outside Africa. 10 African countries were represented. The seminar was the first of its kind in Africa. It provided an opportunity for plant breeders and geneticists from African countries to become acquainted with the potential and the technology of using induced mutations in plant breeding or to update their knowledge in this field. The programme consisted of review papers by invited experts and reports by African researchers outlining their own work and presenting it for discussion. The host institute provided the most appropriate background for the seminar and participants appreciated the opportunity to learn about IITA’s ambitious programmes assisting agricultural development in Africa and focusing upon genetic improvement for major crop plants such as cereals, pulses, roots and tuber crops. Visits were arranged also to the National Cereals Research Institute and the National Horticultural Research Institute, where seminar participants could learn more about crop production in Nigeria and related research. Following extended discussions throughout the seminar, the participants came finally to the following conclusions and recommendations:

1. The Regional Seminar

The programme of the seminar was designed to include discussion on crops grown in Africa, which are being subjected in varying degrees to genetic improvement. Successes, failures and limitations of mutation breeding were demonstrated and principles were outlined that must be followed to have a reasonable chance of success. There was an atmosphere of free expression and interaction among participants, which encouraged profitable exchanges and comments and led to a realistic appreciation of the use of mutation breeding.

The seminar participants reached a consensus that there is a place for mutation breeding in Africa to complement conventional breeding methods and that mutation induction could be a useful tool in creating genetic diversity not available through Germ Plasm Collections.

The seminar has familiarized the participants with the FAO/IAEA Joint Division’s programme and highlighted problems related with and open to approach by mutation breeding in Africa. It emphasized the desirability of cooperation among scientists at both national and international levels and outlined a collective approach to the realization of goals through mutation breeding.

2. Plant improvement in Africa and prospects of utilizing induced mutation techniques

a) Mutation breeding should be considered as an additional useful tool in plant breeding. Its use depends upon the specific objectives chosen for a breeding programme. Its limitations and potential should be weighted in advance against the prospects and costs of other approaches. Mutation breeding often offers best prospects for genetic improvement of apomictic crops and vegetatively propagated plants.

b) Limited resources and personnel available should not be diverted to induced mutation work at the expense of other important plant breeding activities, unless it has clearly been established that mutation breeding offers the better prospects. Resources should primarily be used for important food and cash crops. The economic importance of fibre and oil seed crops for many African countries should not be overlooked. In most circumstances, one would be mistaken to start a mutation induction project before an active breeding programme has been established on the crop species in question.
c) Like in conventional plant breeding programmes, it would be highly desirable to have in mutation breeding projects a multidisciplinary cooperation between geneticists, plant breeders, phytopathologists etc. National institutions in charge of official testing and recommendation of newly developed cultivars should be also aware of mutation breeding programmes from early stages on.

d) Basic elements for success in mutation breeding are:
- to be familiar with the relevant literature
- to start with the best materials available
- to have or develop good screening techniques
- to have experience with the crop species to be improved
- to work with sufficiently large populations
- not to be so easily discouraged as there is also a good deal of luck in all plant breeding (but luck is linked to hard work).

3. Mutagenic treatments

Few breeding institutes in Africa have access to radiation sources or mutagenic chemicals for mutation induction. Radiation treatments for mutation induction will normally be given by trained specialists. However, when plant breeders intend to use mutagenic chemicals, the personnel also must be aware of the health risk and therefore has to have adequate training.

The IAEA Laboratory at Seibersdorf (Austria) has done considerable work on methods of mutagen treatments. It is recommended that the Laboratory continues research in this field with the aim of perfecting the procedures of mutagen application for both physical and chemical mutagens. Based upon its experiences, the IAEA Laboratory will be in a position to perform such treatments (mainly of seeds) for African plant breeders or give adequate advice on optimal treatment procedures.

For vegetatively propagated plants, which are subject to more stringent phytosanitary regulations than seeds or pollen, and which are also more sensitive to shipment over long distances, the installation and use of local radiation sources, preferably for gamma or x-rays, should be considered. IAEA has assisted member states in Africa in a number of atomic energy projects and would be in a position to let interested parties upon request know, where radiation sources have been supplied in connection with such assistance.

4. Training

The participants recognized that training would be desirable under two aspects:

a) to obtain the necessary technical knowledge for inducing mutations and utilizing mutants for plant improvement.

b) to permit a scientist to obtain a higher academic degree which may enable him to carry out a research programme with responsibility.

Training programmes would be useful at various levels:

a) introductory training courses and study tours to get a general understanding about the potential and limitations of induced mutations and at the same time establish contacts with experienced researchers in this field.

b) in-depth training over a longer period (1-2 years) in using induced mutations for solving a problem.

c) specific training to learn a particular technique or become familiar with recently developed methods (up to 3 months).

The site of training may be decided upon by the following criteria:

a) A theoretical introductory course which does not require extensive use of plant material in different stages and generations can be placed anywhere and could be held at many institutions in Africa.
b) A more practical course utilizing extensively laboratory and greenhouse facilities as well as breeding nurseries and material in the field has to be placed at a site with ongoing diversified projects of high standard, and a good number of qualified teaching staff. There are only few places in the world where such conditions are available.

FAO and IAEA are providing various training opportunities for scientists from their member states, such as 4-6 weeks training courses or individual, longer term fellowship training. Training courses will be announced to the Governments and nominations have to be submitted by the governmental authorities. Likewise, requests for individual training have to come via official channels. Information about the training programmes can be requested from FAO and IAEA. The IAEA Seibersdorf Laboratory has a good reputation for short and long training.

5. Coordination at the national level

In some African countries there is insufficient coordination between different institutions concerned with crop improvement. Therefore, valuable genetic materials resulting from induced mutation programmes may not be utilized, as appropriate, for developing improved crop cultivars.

Genetic material resulting from mutation experiments at the academic level should be handed over to institutions concerned with plant breeding. There appears to be a need to establish communication between mutation research groups and breeders. Promising mutants should be included in advanced generation trials at several locations so that the adaptability and agronomic performance of such mutants be studied at an early state in comparison with material derived from other sources. Advanced breeding material intended for release as a variety should be tested and evaluated by an independent national body not involved itself in breeding.

6. Coordination among scientists in African countries

a) Seminar participants will contact colleagues in their home countries to determine crops and objectives for which induced mutations could make a useful contribution towards genetic improvement. In this way, national projects and working groups may be established which could lead to more effective work and more economic use of resources.

b) Once projects are established, project team leaders might be elected and their names be transmitted to FAO/IAEA together with a description of project objectives, facilities and inputs.

c) International workshops with team leaders from different African countries having established related projects should be arranged by FAO/IAEA for coordination between those projects, for determining the most efficient strategy and for establishing close international cooperation and exchange of experiences.

d) If there are reasons for a country not to be ready for national projects or for joining such international cooperation, these may also be communicated to FAO/IAEA as the organisations may be able to provide particular assistance.

7. Action specifically to be taken by FAO and IAEA

a) African scientists who are working on crop improvement by induced mutations want to hold periodical meetings in various parts of Africa to exchange research information and experiences in the use of induced mutations in crop improvement. Experts from other parts of the world should be invited.

b) African scientists working on crop improvement by induced mutations want to initiate and foster closer cooperation, preferably in the form of working groups of crop plants such as cereals, grain legumes, roots and tuber crops, fiber crops or horticultural crops. The objectives of improvement programmes would be decided upon at the national level. However, coordination regarding approaches and methods would be appropriate at an international level.
c) FAO and IAEA should consider the urgent need for more fellowships to enable personnel from each African member state to acquire basic and/or advanced knowledge in the use of induced mutations for crop improvement.

d) The Mutation Breeding Newsletter issued by the Joint FAO/IAEA Division in Vienna was recognized as a valuable means of communication among scientists of different countries working in the field of plant mutations and mutation breeding. The Newsletter should be made available as in the past free of charge to interested plant breeders in Africa.


Conclusions et recommandations

Introduction

Sur l'invitation de la FAO et de l'AIEA, le séminaire s'est tenu à l'Institut international d'agriculture tropicale, à Ibadan, Nigéria. Quarante trois scientifiques ont participé au séminaire, y compris huit experts invités de pays non africains. Dix pays africains étaient représentés. Ce séminaire était le premier de ce type en Afrique. Il a permis aux spécialistes de l'amélioration des plantes et aux généticiens de pays africains de prendre connaissance des possibilités et des techniques d'application des mutations induites pour l'amélioration des plantes, ou de perfectionner leur information dans ce domaine. Le séminaire s'appuyait sur des mémoires présentés par les experts invités et sur des rapports de chercheurs africains décrivant leurs travaux, présentés en tant que base de discussion. L'Institut qui avait accueilli le séminaire représentait le cadre idéal compte tenu du sujet étudié, et les participants ont apprécié l'occasion qui leur était donnée de découvrir les ambitieux programmes de l'IITA en vue de favoriser le développement agricole en Afrique, qui étaient axés sur l'amélioration génétique des plantes de grande culture telles que les céréales, légumineuses, racines et tubercules. Des visites ont aussi été organisées à l'Institut national de recherche sur les céréales et à l'Institut national de recherche horticole du pays, où les participants au séminaire ont pu s'informer sur la production agricole au Nigéria et sur la recherche s'y rattachant. A l'issue de discussions approfondies pendant tout le séminaire, les participants en sont arrivés aux conclusions et recommandations suivantes :

1. Le séminaire régional

Le programme du séminaire prévoyait des discussions sur les plantes cultivées en Afrique qui font actuellement l'objet, à des degrés divers, de travaux d'amélioration génétique. Au cours de ces discussions on a évoqué les succès, les échecs et les limites rencontrées dans l'application de la mutagenèse et dégagé les principes à appliquer pour avoir des chances raisonnables de réussite. Il a régné au cours du séminaire un climat de franchise et d'émulation favorable à des constatations et à des échanges de vues constructifs, ce qui a permis de dresser un tableau réaliste des possibilités d'application de la mutagenèse.
Les participants au séminaire ont reconnu que la mutagénèse avait sa place en Afrique en tant que méthode complétant les méthodes classiques d'amélioration des plantes et que les mutations induites pouvaient être utiles pour engendrer une diversité génétique qui ne pouvait être obtenue avec les collections de plasma germinal.

Le séminaire a permis aux participants de se familiariser avec le programme de la Division mixte FAO/AIEA et a mis en lumière les problèmes liés à l'application de la mutagénèse en Afrique, et ceux susceptibles d'être traités par cette méthode. Il a souligné l'opportunité d'une coopération entre les scientifiques aussi bien au niveau national qu'au niveau international et il a esquissé une approche collective à la réalisation des objectifs par les méthodes de mutagénèse.

2. Amélioration des plantes en Afrique et perspectives d'application des techniques mutagéniques

a) La mutagénèse devrait être considérée comme un outil additionnel utile pour l'amélioration des plantes. Ses possibilités d'application dépendent des objectifs précis fixés pour un programme d'amélioration. Limites et possibilités de cette solution doivent être évaluées à l'avance et mises en regard des perspectives et des coûts d'autres solutions. La mutagénèse offre souvent les perspectives les plus intéressantes pour l'amélioration génétique des plantes se reproduisant par aponixie ou à multiplication végétative.

b) Cependant, les ressources et le personnel limités dont on dispose ne doivent pas être affectés aux travaux de mutagénèse au détriment d'autres activités importantes d'amélioration des plantes, sauf s'il est clairement démontré que c'est cette méthode qui offre les meilleures possibilités. Les ressources devraient en premier lieu être affectées aux cultures alimentaires et commerciales importantes. A cet égard il importe de ne pas perdre de vue l'importance économique des cultures textiles et oléagineuses pour de nombreux pays africains. Dans la plupart des cas, ce serait une erreur que de vouloir lancer un projet mutagénique avant d'avoir mis sur pied un programme actif d'amélioration pour la variété de plante considérée.

c) Comme pour les programmes classiques d'amélioration, il est particulièrement recommandé, dans les projets d'application de la mutagénèse, de s'appuyer sur une coopération multidisciplinaire entre généticiens, sélectionneurs, phytopathologues, etc. Les institutions nationales responsables des essais officiels et des recommandations relatives aux cultivars nouvellement mis au point devraient aussi être informés des programmes de mutation induite dès les débuts.

d) Pour appliquer avec succès les méthodes mutagéniques, il est essentiel :
- d'être bien informé de la documentation technique existante
- de partir avec les meilleurs matériaux disponibles
- d'avoir ou de mettre au point de bonnes techniques de tri
- d'avoir l'expérience de la variété que l'on veut améliorer
- de travailler sur des populations suffisamment vastes
- et enfin, de ne pas se décourager trop facilement, la chance intervenant pour une bonne part dans toutes les activités d'amélioration des plantes (mais la chance est elle-même souvent une question d'assiduité).

3. Traitements mutagéniques

Seul un petit nombre d'instituts de phytosélection en Afrique ont accès à des sources de rayonnement ou à des mutagènes chimiques pour des travaux dans ce domaine. Il va de soi que les traitements par irradiation seront normalement exécutés par des spécialistes qualifiés. Mais si les spécialistes de la sélection décident d'utiliser des mutagènes chimiques, le
personnel doit aussi dans ce cas être informé des risques biologiques et doit donc avoir reçu une formation adéquate.

Le laboratoire de l'AIEA de Seibersdorf (Autriche) a exécuté des travaux considérables sur les méthodes de traitement mutagénique. Il est recommandé qu'il poursuive ses recherches dans ce domaine en vue de perfectionner les méthodes d'application, aussi bien pour les mutagènes physiques que les mutagènes chimiques. Grâce à son expérience, le Laboratoire de l'AIEA serait en mesure d'exécuter ces traitements (principalement sur des semences) pour le compte de spécialistes africains de l'amélioration, ou de fournir des informations valables sur les procédures de traitement optimales.

Pour les plantes à multiplication végétative, qui sont soumises à des réglements phytosanitaires plus rigoureux que les semences ou le pollen, et qui sont aussi plus vulnérables au transport sur de grandes distances, la mise en place et l'utilisation de sources locales de rayonnement, de préférence à rayons gamma ou à rayons X, devraient être envisagées. L'AIEA a aidé des États membres d'Afrique pour un certain nombre de projets relatifs à l'énergie nucléaire et serait en mesure de communiquer aux parties intéressées, sur demande, les cas où des sources de rayonnement ont été fournies dans le cadre de cette assistance.

4. Formation

Les participants ont reconnu que cette formation serait souhaitable à deux titres :

a) pour permettre d'acquérir les connaissances techniques nécessaires pour l'induction des mutations et pour l'utilisation des mutants en vue de l'amélioration des plantes;

b) pour permettre à un scientifique d'obtenir un titre universitaire plus élevé lui permettre d'exécuter un programme de recherche avec responsabilité.

Il serait utile de disposer de programmes de formation à plusieurs niveaux :

a) des cours de formation d'introduction et des voyages d'étude permettant d'acquérir une compréhension générale des possibilités et limites en ce qui concerne les mutations induites et, en même temps, d'établir des contacts avec des chercheurs expérimentés dans ce domaine;

b) une formation approfondie d'une durée plus longue (un ou deux ans) sur l'application des mutations induites pour résoudre un problème donné;

c) une formation spécifique pour des candidats qui devraient acquérir une technique particulière ou se mettre au courant des méthodes récemment mises au point (jusqu'à trois mois).

Le lieu de la formation serait déterminé en fonction des critères suivants :

a) dans le cas d'un cours d'introduction théorique n'exigeant pas de disposer de grandes quantités de plantes à différents stades et de différentes générations, cette formation pourrait avoir lieu n'importe où, et pourrait se tenir dans de nombreux établissements en Afrique;

b) pour les cours à caractère plus pratique exigeant des moyens importants de laboratoire et de culture en serre, ainsi que l'accès à des pépinières d'amélioration et à des matériaux sur le terrain, il faudrait choisir un lieu où se poursuivent des activités diversifiées à haut niveau, et où il existe un personnel de formation qualifié en nombre suffisant. Seuls quelques endroits au monde remplissent ces conditions.

La FAO et l'AIEA offrent diverses possibilités de formation pour les scientifiques envoyés par des États membres, par exemple des cours de
formation de quatre à six semaines, ou une formation individuelle de plus longue durée au titre de bourses d'étude. Ces cours de formation seront notifiés aux gouvernements et les nominations devront être présentées par les autorités. De même, les demandes de formation individuelle doivent être transmises par les voies officielles. Des renseignements sur les programmes de formation peuvent être obtenus auprès de la FAO et de l'AIEA. Le Laboratoire de l'AIEA de Seibersdorf jouit d'une bonne réputation pour les stages de formation de courte durée et de longue durée.

5. Coordination au niveau national

Dans certains pays africains, il existe une coordination insuffisante entre les diverses institutions s'occupant de l'amélioration des cultures. De ce fait, il peut arriver que des matériaux génétiques utiles issus de programmes de mutagenèse ne soient pas utilisés comme il le faudrait pour la mise au point de cultivars améliorés.

Les matériaux génétiques provenant d'expériences de mutations au niveau universitaire devraient être transmis aux institutions s'occupant d'amélioration des plantes. Il apparaît nécessaire d'établir une communication entre les groupes de recherche sur les mutations et les sélectionneurs. Les mutants potentiellement intéressants devraient être soumis à des essais de génération au stade avancé en plusieurs emplacements pour que l'on puisse étudier dès les débuts leur adaptabilité et leurs performances agronomiques par comparaison avec celles de matériaux provenant d'autres sources. Les matériaux ayant atteint un stade de mise au point avancé, destinés à être mis en exploitation comme variétés, devraient être essayés et évalués par un organe national indépendant ne participant pas lui-même aux activités d'amélioration des plantes.

6. Coordination entre scientifiques dans les pays africains

a) Les participants au séminaire prendront contact avec des collègues dans leurs pays d'attache pour déterminer les cultures, et les objectifs, pour lesquels les techniques de mutagenèse pourraient utilement contribuer à l'amélioration génétique. Il sera ainsi possible d'établir des projets nationaux et des groupes de travail qui devraient permettre un travail plus fructueux et une mise en œuvre plus économique des ressources.

b) Une fois les projets mis sur pied, des chefs d'équipe de projet pourraient être élus, dont les noms seraient communiqués à la FAO/AIEA, avec une description des objectifs, moyens et entrées du projet.

c) Des groupes de travail internationaux où participeraient les chefs d'équipe de différents pays africains ayant instauré des projets apparentés devraient être organisés par la FAO/AIEA en vue d'assurer la coordination entre ces projets, de déterminer la stratégie la plus efficace, et d'entretenir une coopération et un échange d'expérience étroits au niveau international.

d) Dans le cas où pour certaines raisons, un pays ne serait pas prêt à entreprendre des projets nationaux, ou à se joindre à cette coopération internationale, il pourrait aussi en informer la FAO/AIEA, ces organisations pouvant alors, selon le cas, lui fournir une assistance particulière.

7. Action qui incomberait particulièrement à la FAO et à l'AIEA

a) Les scientifiques africains travaillant dans le domaine de l'amélioration des cultures par mutations induites souhaitent tenir des réunions périodiques dans diverses parties de l'Afrique pour procéder à des échanges d'information et d'expérience sur la recherche dans l'application des mutations induites pour l'amélioration des cultures. Les experts d'autres parties du monde devraient être invités.
b) Les scientifiques africains travaillant dans le domaine de l’amélioration des cultures par mutations induites souhaitent lancer et entretenir une coopération plus étroite, de préférence dans le cadre de groupes de travail sur les cultures telles que céréales, légumineuses à grains, racines et tubercules, plantes textiles ou plantes horticoles. Les objectifs des programmes d’amélioration seraient décidés au niveau national. Cependant, une coordination en ce qui concerne les approches et les méthodes serait indiquée au niveau international.

c) La FAO et l’AIEA devraient prendre en compte le besoin urgent de créer un plus grand nombre de bourses d’étude pour permettre au personnel de chaque État membre africain d’acquérir des connaissances de base et/ou poussées sur l’application de la mutagenèse pour l’amélioration des plantes.

d) La Mutation Breeding Newsletter publiée par la Division mixte FAO/AIEA à Vienne a été considérée par le séminaire comme un moyen utile de communication entre les scientifiques de différents pays travaillant dans le domaine des mutations des plantes et de l’amélioration par mutagenèse. Cette publication devrait comme par le passé être communiquée gratuitement aux spécialistes de l’amélioration des plantes intéressés en Afrique.
Since 1969, the Joint FAO/IAEA Division undertakes to collect and publish information on varieties of crop plants that were developed directly from induced mutants or by using mutants in cross breeding (Micke 1972 and Sigurbjörnsson and Micke 1969, 1974). The purpose of this undertaking is to assess realistically the potential of induced mutation techniques to contribute towards progress in plant breeding. Varieties which have successfully passed official trials and were approved or recommended by national governmental authorities for cultivation, appear to be good indicators of practical success. By 1 October 1978, we know about 195 of such varieties in agricultural crop plants (Table 1). They belong to 37 different plant species and come from 30 different countries (Table 2). In addition, there are more than 120 mutant cultivars of ornamental plants known, which represent a considerable economic value for countries with developed horticulture (Broertjes and van Harten 1978).

More important than the number of varieties, of course, would be to know more about the commercial value that could be attributed to the mutated traits. To determine this is a rather unusual and difficult task. A variety performance is based upon all the genes that make up the genotype, upon complex interactions of these genes within the genotype and with the environment in the broadest sense. Attempts have, nevertheless, been made to assess the commercial value of individual mutant varieties (e.g., Bossi, these proceedings; Sigurbjörnsson and Micke 1969, 1974; Micke 1973, 1977) which allow the conclusion that a successful mutation breeding, in general, is a profitable enterprise. Of course, success is bound to a number of conditions, such as:

- clearly identified objective
- adequate selection methods
- sufficiently large populations
- qualified personnel experienced in breeding the crop species in question

It is a popular opinion that through mutation breeding certain objectives can be reached faster than through cross breeding. This is true to some extent but it depends, of course, on the objective and the genetic resources available. Table 3 demonstrates that improved mutant varieties were released within less than 10 years including several years of official testing, whereas, developing a new variety by cross breeding usually requires 10 – 15 years.

The majority of varieties developed directly from induced mutants was released in the early seventies (Table 4). This observation may
correspond to the relatively widespread interest in experimenting with radiation during the sixties. The number of varieties originating from cross breeding programmes with induced mutants appears to increase drastically in recent years. Most of the mutant parents used in these programmes had proven their agronomic and economic value as released varieties before they were included in crosses. Therefore, we are observing at present a second wave of results from the earlier popularity of mutation research.

It can be expected, that from now on mutation induction and the use of induced mutants in cross breeding will be looked at more realistically ("sine ira et studio") as being a valuable and useful additional tools for plant breeding.

References


## Table 1. List of Mutant Varieties of Agricultural Crop Plants

<table>
<thead>
<tr>
<th>Species and Name of Variety</th>
<th>Country and Year of Release</th>
<th>Mutagen and Year of treatment</th>
<th>Mutant cross?</th>
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Hordeum sativum (contd.)

Balder, Fin 1960 \( \times \) 1946
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Mari (mat-a5) Sweden 1962 \( \times \) 1949
Pennrad USA 1963 Nth 1956
Alassch FRG 1963 \( \times \) cross
Gamma 4 Japan 1965 gamma 1958
Diamant CSSR 1965 \( \times \) 1956
Milne Golden Promise UK 1966 gamma 1956
Amei FRG 1966 \( \times \) cross
Helias Sweden 1966 \( \times \) 1946 cross
Nirasaki Nijo 8 (Gamma No. 8) Japan 1967 gamma 1958 cross 1962
Luther USA 1967 DES 1960
Betina France 1968 EMS
Bonneville 70 USA 1969 Nth 1952
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Amagi Nijo 1 Japan 1971 \( \times \) 1965
KDB-1 India 1972 \( \times \) 1960
Sva Sweden 1972 \( \times \) 1949 cross
Rupal Sweden 1972 \( \times \) 1946 cross
Ametyst (HE 464b) CSSR 1972 \( \times \) 1956 cross
Favorit (HE 481) CSSR 1973 \( \times \) 1956 cross
Hana (HE 493) CSSR 1973 \( \times \) 1956 cross
Trumpf GDR 1973 \( \times \) 1956 cross
Fuji 2-jyo II Japan 1974 BUDR + gamma
Blazer (WA6704-62) USA 1974 Nth 1962 cross
Minsk USSR 1974 gamma
Radiation Korea 1974 Nth 1967
Boyer (WA Sel. 1094-67) USA 1974 DES 1960 cross
Senat Sweden 1974 \( \times \) 1946 cross
Salve Sweden 1974 \( \times \) 1949 cross
Pakel USSR 1975 \( \times \) 1966
Hankkija's Aapo (Hja 4003) Finland 1975 \( \times \) 1961
Hankkija's Eero (Hja 4715) Finland 1975 \( \times \) 1949 cross
Nadja GDR 1975 \( \times \) 1956 cross
Goldespear UK 1975 gamma 1956 cross
Deam USA 1975 Nth 1952 cross
Goldmarker UK 1976 gamma 1956 cross
Markeli 5 Bulgaria 1976 gamma 1967
Atlas CSSR 1976 gamma 1956 cross
Rapid CSSR 1976 \( \times \) 1956 cross
Jupiter UK 1976 EMS, \( \times \) 1956 cross
Minak UK 1976 EMS, \( \times \) 1956 cross
Twen USSR 1976 NTH 1966
Atlanta Canada 1977 \( \times \) 1945 cross
Spartan CSSR 1977 \( \times \) 1956 cross
Alf Denmark 1978 Nth 1969
Stange Norway 1978 \( \times \) 1949 cross
Safir CSSR 1978 \( \times \) 1956 cross

Lactuca sativa

Evergreen Japan 1967 beta + gamma 1959
Giantgreen Japan beta

Linum usitatissimum

Redwood 65 Canada 1965 \( \times \) 1951
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<tr>
<td><strong>Solanum tuberosum</strong></td>
<td>Konkei No. 45</td>
</tr>
<tr>
<td><strong>Solanum khasianum Clarke</strong></td>
<td>RRL-20-2</td>
</tr>
<tr>
<td><strong>Trifolium incanutum</strong></td>
<td>Cardinal</td>
</tr>
<tr>
<td><strong>Trifolium subterraneum</strong></td>
<td>Univager</td>
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</table>
### Triticum aestivum

<table>
<thead>
<tr>
<th>Variety</th>
<th>Country</th>
<th>Year</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elsa</td>
<td>FRG</td>
<td>1960</td>
<td>X</td>
</tr>
<tr>
<td>N P 836</td>
<td>India</td>
<td>1961</td>
<td>X</td>
</tr>
<tr>
<td>Sinalococho Gama</td>
<td>Argentina</td>
<td>1962</td>
<td></td>
</tr>
<tr>
<td>Lewis</td>
<td>USA</td>
<td>1964</td>
<td></td>
</tr>
<tr>
<td>Stadler</td>
<td>USA</td>
<td>1964</td>
<td></td>
</tr>
<tr>
<td>Austro Kolben</td>
<td>Austria</td>
<td>1966</td>
<td>X</td>
</tr>
<tr>
<td>Sharbati Sonora</td>
<td>India</td>
<td>1967</td>
<td>gamma</td>
</tr>
<tr>
<td>Sirius</td>
<td>FRG</td>
<td>1968</td>
<td>X</td>
</tr>
<tr>
<td>Zenkouzi-Komugi</td>
<td>Japan</td>
<td>1969</td>
<td>gamma</td>
</tr>
<tr>
<td>Novosibirskaja 67</td>
<td>USSR</td>
<td>1969</td>
<td></td>
</tr>
<tr>
<td>Pusa Lerma</td>
<td>India</td>
<td>1971</td>
<td>gamma</td>
</tr>
<tr>
<td>Odessaia 75</td>
<td>USSR</td>
<td>1975</td>
<td>X</td>
</tr>
<tr>
<td>Odesskaia polukarlikovaja</td>
<td>USSR 1975</td>
<td>NmH</td>
<td></td>
</tr>
<tr>
<td>Polukarlikovaya-49</td>
<td>USSR</td>
<td>1978</td>
<td>NmH</td>
</tr>
<tr>
<td>Taava</td>
<td>Finland</td>
<td>1978</td>
<td>gamma</td>
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### Triticum turgidum spp durum

<table>
<thead>
<tr>
<th>Variety</th>
<th>Country</th>
<th>Year</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castelporziano</td>
<td>Italy</td>
<td>1968</td>
<td>Nth</td>
</tr>
<tr>
<td>Castelfusano</td>
<td>Italy</td>
<td>1968</td>
<td>Nth</td>
</tr>
<tr>
<td>Casteldelmonte (GRA 145)</td>
<td>Italy</td>
<td>1970</td>
<td>Nf</td>
</tr>
<tr>
<td>Creso (FB 55)</td>
<td>Italy</td>
<td>1974</td>
<td>X</td>
</tr>
<tr>
<td>Castelnuovo (CaB 125)</td>
<td>Italy</td>
<td>1975</td>
<td>X</td>
</tr>
<tr>
<td>Mida (FB 59)</td>
<td>Italy</td>
<td>1975</td>
<td>X</td>
</tr>
<tr>
<td>Tito (FC 108)</td>
<td>Italy</td>
<td>1975</td>
<td>Nth</td>
</tr>
<tr>
<td>Augusto</td>
<td>Italy</td>
<td>1976</td>
<td>Nth</td>
</tr>
<tr>
<td>Miredur</td>
<td>Austria</td>
<td>1978</td>
<td>Nth</td>
</tr>
</tbody>
</table>

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**Table 2. Number of mutant varieties of agricultural crop plants released in different countries**

(As of 1 October 1978)

<table>
<thead>
<tr>
<th>Country</th>
<th>Varieties</th>
</tr>
</thead>
<tbody>
<tr>
<td>India</td>
<td>29</td>
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<tr>
<td>USA</td>
<td>23</td>
</tr>
<tr>
<td>USSR</td>
<td>18</td>
</tr>
<tr>
<td>Japan</td>
<td>17</td>
</tr>
<tr>
<td>Sweden</td>
<td>15</td>
</tr>
<tr>
<td>CSSR</td>
<td>11</td>
</tr>
<tr>
<td>Italy</td>
<td>9</td>
</tr>
<tr>
<td>Republic of China</td>
<td>8</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>6</td>
</tr>
<tr>
<td>Federal Republic of Germany</td>
<td>6</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>6</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Country</th>
<th>Varieties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canada</td>
<td>6</td>
</tr>
<tr>
<td>Australia</td>
<td>3</td>
</tr>
<tr>
<td>Philippines</td>
<td>3</td>
</tr>
<tr>
<td>GDR</td>
<td>3</td>
</tr>
<tr>
<td>Republic of Korea</td>
<td>3</td>
</tr>
<tr>
<td>Argentina</td>
<td>3</td>
</tr>
<tr>
<td>Austria</td>
<td>3</td>
</tr>
<tr>
<td>Netherlands</td>
<td>2</td>
</tr>
<tr>
<td>Burma</td>
<td>2</td>
</tr>
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</table>

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Table 3. **TIME USED TO DEVELOP MUTANT VARIETIES**

<table>
<thead>
<tr>
<th>No. of years</th>
<th>Up to 4</th>
<th>5-6</th>
<th>7-8</th>
<th>9-10</th>
<th>11-12</th>
<th>13-14</th>
<th>15-16</th>
<th>17-18</th>
<th>19-20</th>
<th>21-25</th>
<th>&gt; 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>rice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>direct</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>cross</td>
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<td>2</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>barley</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>direct</td>
<td>2</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cross</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>9</td>
<td>7</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>other annual</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>crop plants</td>
<td>direct</td>
<td>1</td>
<td>6</td>
<td>8</td>
<td>10</td>
<td>8</td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>cross</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td></td>
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</tr>
</tbody>
</table>
Table 4. MUTANT VARIETIES ACCORDING TO YEAR OF RELEASE

<table>
<thead>
<tr>
<th>Varieties developed through direct multiplication of selected mutant</th>
<th>before 1955</th>
<th>1955-59</th>
<th>1960-64</th>
<th>1965-69</th>
<th>1970-74</th>
<th>1975-78</th>
</tr>
</thead>
<tbody>
<tr>
<td>seed propagated crops</td>
<td>3</td>
<td>6</td>
<td>14</td>
<td>24</td>
<td>33</td>
<td>19</td>
</tr>
<tr>
<td>vegetatively propagated crops (without ornamentals)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Varieties developed through cross with mutant(s)</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>9</td>
<td>19</td>
<td>32</td>
</tr>
</tbody>
</table>
LIST OF PARTICIPANTS

A.M.T. Abo-Hegazi
RadioMology Department
Atomic Energy Establishment
Cairo, Egypt

G. Acquaah
Department of Crop Science
University of Ghana
Legon, Accra, Ghana

M.A. Adenihun
National Cereals Research
Institute
Moor Plantation
Ibadan, Nigeria

G. Ahnström
University of Stockholm
Wallenberg Laboratory
10691 Stockholm, Sweden

K. Alluri
Cereal Improvement Programme
I.I.T.A.
P.M.B. 5320
Ibadan, Nigeria

V. Appiah
Ghana Atomic Energy Commission
Box 80
Legon, Ghana

V.L. Asnani
Cereal Improvement Programme
I.I.T.A.
P.M.B. 5320
Ibadan, Nigeria

A. Boncoula
I.N.R.A.N.
B.P. 429
Niamey, Niger

O. Botorou
Centre National de Recherches
Agronomiques de Tarna
B.P. 240
Maradi, Niger

I.W. Buddenhagen
Cereal Improvement Programme
I.I.T.A.
P.M.B. 5320
Ibadan, Nigeria

C.K. Bulungu
Kings College Budo
P.O. Box 7121
Kampala, Uganda

G. Burton
Research Geneticist
SEA-AR
USDA Coastal Plain Station
Tifton
Ca. 31794, U.S.A.

M. Dabi
Direction de l'Agriculture
Station Agronomique de Deli
B.P. 26
Moundou, Tchad

R.B. Dadson
Department of Crop Science
University of Ghana
Legon, Accra, Ghana

L. Denton
National Horticultural Research
Institute
P.M.B. 5432
Idi-Ishin
Ibadan, Nigeria

B. Deru
Department of Biological Sciences
University of Lagos
Lagos, Nigeria

A. Gustafsson
Institute of Genetics
Solvegatan 29
22362 Lund, Sweden

S.K. Hahn
Root & Tuber Improvement Programme
I.I.T.A.
P.M.B. 5320
Ibadan, Nigeria

M.S. Haq
Faculty of Agronomy
University of Zulia
Maracaibo, Venezuela

H.A. Hussein
Faculty of Agriculture
University of Cairo
Giza, Egypt

D. Jagathesan
Sugarcane Breeding Institute
Coimbatore 641007, India

Z.L.M. Kanyeka
A.R.I.
Katrin Ifakara, Tanzania

H. Khalifa
A.R.C., Cotton Breeding Section
P.O. 30, Khartoum North, Sudan

I. Magah
CNRA - Tarna
B.P. 240
Maradi, Niger

J. Monyo
FAO
Via delle Terme di Caracalla
00100 Rome, Italy
OPENING CEREMONY OF SEMINAR

From right to left:
Dr. A.C. Obi, National Science and Technology Development Authority, Ibadan, Nigeria.

Dr. W.K. Gamble, Director-General, International Institute of Tropical Agriculture, Ibadan, Nigeria.

Dr. J.H. Monyo, Head, Research Development Centre, FAO, Rome, Italy.

Dr. A. Micke, Scientific Secretary, FAO/IAEA Joint Division, Vienna, Austria.